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**BIOLOGY OF THE PARASITOIDS AND
PREDATORS OF *TRIBOLIUM* SPP.
(COLEOPTERA: TENEBRIONIDAE)**



**THESIS SUBMITTED FOR THE DEGREE
OF
MASTER OF PHILOSOPHY
IN THE
INSTITUTE OF BIOLOGICAL SCIENCES
RAJSHAHI UNIVERSITY, RAJSHAHI 6205
BANGLADESH**

**by
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B Sc (Hons) M Sc (Rajshahi)**

April 2004

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Bangladesh**

*Dedicated
to
My Beloved
Parents*

Declaration

I do hereby declare that the work submitted as a thesis entitled **Biology of the Parasitoids and Predators of *Tribolium* spp. (Coleoptera: Tenebrionidae)** in the Institute of Biological Sciences, Rajshahi University, for the degree of Master of Philosophy is the result of my own investigation carried out under the supervision of Dr Md Wahedul Islam, Professor, Institute of Biological Sciences, Rajshahi University. The thesis has not been currently submitted for any other degree.

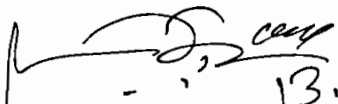
April 2004
Rajshahi

Farhana Rahman
Farhana Rahman 13.04.04

Certificate

This is to certify that the contents reported in this thesis **Biology of the Parasitoids and Predators of *Tribolium* spp. (Coleoptera: Tenebrionidae)** are original works carried out by Farhana Rahman under my supervision for the degree of Master of Philosophy. It contains no material previously published or submitted for any other degree.

Supervisor



13.4.04

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Abstract

Two parasitoids and one predator were recovered from the red flour beetle, *Tribolium castaneum* (Herbst) and the confused flour beetle, *T. confusum* Duval infested wheat flours. The larval-pupal parasitoids are *Rhabdopyris zae* Waterston and *Holepyris sylvanidis* Brethes (Hymenoptera: Bethyridae) and the predator is *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae). Some aspects of biology of these parasitoids and predator were investigated in the laboratory condition on the above two species of *Tribolium*.

R. zae although a larval-pupal ectoparasitoid showed a decided preference towards the fourth instar larvae and early pupae of the host. The developmental time varied in the male and female adult parasitoids. The male and female of *R. zae* completed development within 19.06 ± 0.59 and 20.14 ± 0.58 days in *T. castaneum* while in *T. confusum* it was 20.10 ± 0.77 and 21.20 ± 0.92 days, respectively at $30 \pm 1^\circ\text{C}$ and 70% R. H. The males are polygamous but the females are always monogamous. Both the sexes of *R. zae* lived maximum days when host was supplied continuously. The female deposited maximum number of eggs when hosts were abundant. The sex ratio of *R. zae* always showed female biased and dependent on the size of the host.

H. sylvanidis is also a primary larval-pupal ectoparasitoid of *T. castaneum* and *T. confusum*. After emergence the male took the initiative in mating and courtship. The fourth instar larvae were preferred for oviposition. The male of *H. sylvanidis* completed life cycle within 15.50 ± 0.62 and 16.00 ± 0.47 days in *T. castaneum* and *T. confusum* whereas the female required 16.66 ± 0.72 and 17.15 ± 0.80 days. Females are monandrous while males are polygynous. The hosts fluids were necessary for greater longevity in both the sexes. *H. sylvanidis* is arrhenotokous.

Xylocoris flavipes (Reuter) is a predator of different larval and pupal stages of *Tribolium*. The early stage was most preferred than that later stages. The male of *X. flavipes* completed life cycle within 16.99 ± 1.86 and 17.80 ± 1.30 days in *T. castaneum* and *T. confusum* whereas the female required 16.00 ± 1.63 and 17.00 ± 1.63 days. The female biased sex ratio of *X. flavipes* was noticed.

Preface

Since the dawn of civilization man has been in constant strife with insect pests competing for food, fibre and shelter. To the change of systems of living and culture, man has developed new measures and techniques for survival. Considering the future demand man began to store food (Metcalf and Flint 1962). In spite of our best efforts world crop loss due to pests is approximately 35% of the total production each year (McEwen 1978, Pimental 1978). This reduction is further increased by post-harvest losses caused by insects and other pests (Wright 1976).

Stored-product insects are generally considered to cause 5-10% losses in the world (Burkholder 1990). But loss in the sub-tropical regions is higher than in the temperate climate zones. However, loss of 20% or more may occur in tropical countries through insect attack after harvest (Mondal and Port 1994), because the climate and storage conditions in the tropical countries are highly favourable for insect growth and development. In Bangladesh this loss ranges from 10 to 15% (Hussain 1996). The loss of food grain during storage due to insect pest is a serious problem. Both contamination and substantial economic losses to the presence of pest insects in a stored product sustain to loss the product and decrease in nutritional value (Wilbur and Mills 1985, Burkholder and Faustini 1991). Thus, the need for conservation of grains from infestation caused by pests is most necessary.

Among the stored-product insect pests, the flour beetle *Tribolium* has long been recorded as serious pests of stored grains and cereals (Chittenden 1896), and widely distributed throughout the world (Sokoloff 1972). The ability of both adults and larvae to exploit a huge number of stored products (Ziegler 1977) has contributed to their status as major pests (Good 1936).

Different protective measures have been applied for the control of *Tribolium*. Out of them chemical insecticides is the most conventional. Large scale use of chemical

insecticides in controlling the insect pests has several drawbacks including environmental pollution, multiresistance to pesticides and loss of wildlife including pollinator and economically beneficial insects (Munakata 1977, Pimental 1983 and Georghiou and Mellan 1983). Moreover, the use of chemical insecticides in stored grain insects leads to problems of undesirable residues. All these factors have paved the way to find new strategies for control of *Tribolium*.

In recent years, biological suppression of stored-product insect pests has been recognized as an acceptable strategy. Various parasitoids and predators of storage pests have considerable potential for providing requisite pest management alternatives for post-harvest agriculture. *Tribolium* is parasitized in their life stages by some parasitoids and predators (Arbogast 1975, Ahmed and Islam 1988). These parasitoids and predators generally occur in the natural store in humid months of the year and they considerably check the population of *Tribolium*. Scientists are now in intensive search for the parasitoids and predators of *Tribolium* as an alternative to other methods of pest control. Before releasing the parasitoids and predators in the store for biological control programme, a through knowledge is required about their biology. So far, serious attempt has not been conducted on the parasitoids and predators of *Tribolium* in Bangladesh. The objective of the present study is to observe some aspects of their biology to achieve this goal.

Acknowledgement

I feel immense pleasure to record my deep sense of sincere gratitude to my honourable teacher and supervisor Dr Md Wahedul Islam, Professor, Institute of Biological Sciences, Rajshahi University for his proper guidance, valuable suggestions and constructive criticism during the course of the present investigation.

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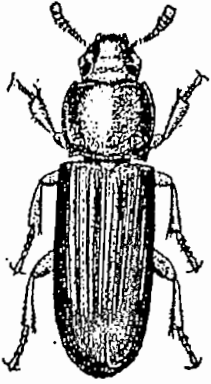
I wish to express my warm gratefulness to Dr Khandaker Nesar Ahmed, Senior Scientific Officer, Bangladesh Council of Scientific and Industrial Research (BCSIR), Rajshahi and Professor Dr Bidhan Chandra Das, Department of Zoology, Rajshahi University for their inspiration and valuable advice during the course of this work.

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Lastly but by no means the least, I owe a deep debt of gratitude to my husband M Aftab Hossain, Scientific Officer, Bangladesh Atomic Energy Commission, Dhaka for constant moral support, sacrifice and inspiration.

The author



Chapter 1

Introduction

Introduction

More than 2000 species of field and storage pests annually destroy approximately one third of world's food production, valued about US \$100 billion among which highest losses (43 % of potential production) occur in developing Asian countries (Ahmed and Grainge 1986). Storage losses from insect attack are often as great as those sustained by the growing crops. Crop losses due to insects have been estimated to amount up to 50% of total agricultural output (Shani 1998), and annual post-harvest losses resulting from insect damage, microbial deterioration and other factors are estimated to be 10-25% of production world wide (Mathews 1993). In USA and Canada, 20-26% of the stored wheat was infested by insect pests (White *et al.* 1985). In India 6.5% stored grains losses are caused by insects (Raju 1984).

Bangladesh is an agricultural country and her economy mainly depends on agricultural production. Huq (1980) reported 2.6% average farm-level storage loss in the weight of unhusked rice in 3-6 months due to insect's infestation. Greeley (1980) reported yearly losses of 3.3, 2.4 and 2.5% for stored dry raw paddy, parboiled paddy and parboiled rice respectively, by insect pests in Bangladesh.

A large number of beetles and weevils attack stored products. Among them *Tribolium* is a major pest and distributed throughout the world (Good 1936, Cotton 1947, Pruthi and Singh 1950). The genus *Tribolium* includes about 30 species of which *Tribolium castaneum* (Herbst), *T. confusum* Duval, *T. anaphe* Hinton, *T. audex* Halstead, *T. destructor* Uyttent, *T. freemani* Hinton and *T. brevicornis* Lec. are pests of primary and secondary importance (Sokoloff and Spedding 1980). Some major *Tribolium* spp. associated with stored products are shown in Table 1.

Table 1. Some major *Tribolium* spp. associated with stored products (Sokoloff 1972, 1974).

Species (Common names)	Origin/Distribution	Products infested
<i>Tribolium castaneum</i> (Herbst) (Red flour beetle)	Originated in India and worldwide distribution	Grains, flour cereal and cereal products, groundnuts, dried fruits and occasionally peas and beans
<i>Tribolium confusum</i> Duval (Confused flour beetle)	Originated in Ethiopia, distribution extends further north than that of <i>T. castaneum</i> , less frequent in the tropics.	Grains, flour cereal and cereal products
<i>Tribolium destructor</i> Uyttent (Dark flour beetle)	Europe, subtropical region and cool areas in the tropics, e.g. Afghanistan and the high lands of Ethiopia and Kenya	Milled cereals, oilseeds, pulse and dried fruit
<i>Tribolium brevicornis</i> Lec. (Giant flour beetle)	USA	Cereal and cereal products
<i>Tribolium anaphe</i> Hinton	Africa	Cottonseed, cocoa, beans and palm kernels
<i>Tribolium audex</i> Halstead	North America	Cereal and cereal products
<i>Tribolium madens</i> (Charp)	North and Eastern Europe, Portugal, Egypt and USA	Cereal and cereal products
<i>Tribolium freemani</i> Hinton	Originated in India, rediscovered in Japan in 1978	Cereal and cereal Products

The red flour beetle *T. castaneum* and the confused flour beetle *T. confusum* are common pests of indoor storage facilities and processing plants (Arthur 2000). Both are well known stored product pests having cosmopolitan distribution (Okumura and Strong 1965, Sokoloff 1972, 1974). They are generally known among millers as 'bran bugs'. *T. castaneum* contaminate more than they consume.

According to Khan and Mannan (1991) this contamination results from the presence of living or dead insects or insect parts, cast exuviae, egg shell and pupal cases, fecal and persistent odour and webbing food.

It is not clearly known when the beetles began their grain dwellings habit. Wilbur and Mills (1978) reported these flour beetles being associated with grain stored at least since early Egyptian times. Specimen of *Tribolium* were found in a Pharaonic tomb of about 2500 B.C. when commerce was largely restricted to the Mediterranean region and Southern Asia (Andres 1931). According to Blair (1930), *T. castaneum* was commonly found in India as wild state. It was also found in North America and elsewhere but not at all commonly. Hinton (1948) considered it to be a potential pest in the tropics. *T. confusum* originated in Ethiopia in 1962 (Mondal 1994) and its distribution extended further north than that of *T. castaneum* but less frequent in the tropics. Almost without exception, *Tribolium* originally lived in nests of bees and occasionally under the bark of trees and in rotting logs. Later on they adopted the flour feeding habit (Good 1933, 1936). Furthermore the advanced hypothesis was that these beetles were originally herbivorous, feeding primarily on carbohydrates, fungi and other materials of plant origin. Under natural conditions they can survive as scavengers or predators of social insects. The cannibalistic habit of *Tribolium* has led to another hypothesis that flour beetles originally were omnivorous surviving in nature as scavengers or semi-predators (Muller and Sokoloff 1982).

Tribolium beetles live in numerous stored product commodities. Both adults and larvae exploit a wide variety of dried vegetable products that contributed their status as major pests (Ziegler 1977). The beetles are unable to feed on whole cereal grains because

their mouthparts are not adapted for chewing large and hard pieces of food. They live on flour, cracked grain or breakfast food or meal is found in rice, wheat flour, cornmeal, barley flour and oatmeal (Chittenden 1896, 1897). They also feed on chocolate, spices, peppers, peas, oilseeds, semolina, coffee, cocoa, beans, and various kinds of nuts and sometimes feed on specimen in insect collection (Good 1933). Some cereal products are more infested than others (Chapman 1918, Shepard 1940, Smallman and Laschiavo 1952, Magis 1954).

The long lifespan and long reproductive period enable *Tribolium* spp. to spend a considerable period searching for new food sources (Dawson 1977). *Tribolium* larvae and adults are highly efficient cannibals of eggs and pupae (Ryan and Park 1970).

According to Good (1933) the length of the life cycle varies depending on the food, humidity and temperature. The cycle become longer as the temperature fall. Chapman (1931) reported that adult *Tribolium* die with in a few weeks if subjected to a temperature as low as 7°C. The lifespan of adult *Tribolium* ranges from 3 months to a year and eight months but sometimes it may be more than three years (Good 1936). *T. castaneum* possesses in its life cycle egg, larval, pupal and adult stages. The egg is white, covered with sticky substances so that soon covered with flour media. The eggs hatch into small larvae. The incubation period of the eggs is 4-5 days. The colour of larvae is yellowish-white and measures 1 mm in length. As it matures, it turns reddish yellow, becomes hairy and measures over 6 mm in length. Its head, appendages and the last abdominal segment are darker. The adult is a small reddish-brown beetle, measuring about 3.5 mm in length and 1.2 mm in width. Its antennae are bent and bear a distinct club formed by the three enlarged terminal joints. The last antennal segment is transversely rounded.

Much of the damage done *T. castaneum* and *T. confusum* is directly to the kernels (germ and endosperm). Their feeding and metabolic activities alter the colour of the flour into a pinkish colour, with an offensive odour and a disgusting taste. The flour is said to be conditioned (Chittenden 1896). This also adversely affects the viscous and

elastic properties of the flour without creating a disgusting taste (Payne 1925). The flour is also conditioned by a longstanding culture of *Tribolium* due to the presence of living or dead insects or insect parts, cast exuviae, egg shells, pupal cases, faecal matter, noxious and persistent odours and webbing of food (Mondal 1983, 1985, Khan and Mannan 1991).

Control of *Tribolium*

In Bangladesh, *T. castaneum* and *T. confusum* are abundantly found associated with stored grain of different cereals (Alam 1971). It is found in almost every kind of stored grain and their products. A large number of investigators worked separately on different parameters of IPM in Bangladesh to control *T. castaneum*. But unfortunately no sufficient information or work has been done on this pest to control it by using parasitoids and predators. However, the present research was designed to investigate the effectiveness of parasitoids and predators to control *T. castaneum* and *T. confusum*.

Different control measures are considered for stored pests, such as, mechanical control, physical control, chemical control, biological control, etc.

Physical control

There is a renewed interest in the application of physical control methods against storage pests because consumers are becoming more aware of the consequences of immoderate use of chemical pesticides in the food chain. Various physical control practices have been reviewed by a number of researchers (Shejbal 1980, Armitage 1987, Lessard 1987, Navarro and Jay 1987). Physical control methods give immediate tangible results and are generally popular with, and convincing to farmers. However, it must be stressed that much research is still needed before any of the physical techniques recommended for pest control. Several physical control measures are available and these are:

1. High- temperature fluidized beds have reached a pilot scale for the disinfestation of grains and their products. It has been confirmed that short exposures to temperature above 60°C are generally effective for the disinfestation of grains (Evans 1981,1987). Evan's method is based on fluidized-bed drying techniques (Dermott and Evans 1978) and permits rapid heating of individual grains. In tropical countries solar dryers provide an opportunity for disinfestation by the heat of the sun.
2. Stored dried fruits are protected by keeping them in refrigerated warehouses in different parts of the world. The multiplication of pests in grains is slowed or stopped by using mobile refrigeration units for grain elevators or by chilled-air ventilation of stores. Cooling and drying methods in storage pest management are discussed by Evans (1987).
3. Controlled and modified atmosphere storage practice for protecting grains have developed greatly during recent years. Much information on these subjects is now available (Hyde *et al.* 1973, Anonymous 1984, Evans 1987).
4. Dry foodstuffs can be disinfested by exposing them to non-ionizing electromagnetic energy, microwaves or high-frequency dielectric heating. The same procedures can be applied to packaged goods.
5. Ionizing radiation such as X-rays and γ -rays allow sterilization at lower dosage but are lethal at higher doses. The application of ionizing radiation in controlling storage pests.

Chemical control

The use of chemical pesticides is still the chief method of control for insect and mite pests of stored products. Many study have been conducted for the control of *Tribolium* spp. by conventional chemical insecticides (Topozeda *et al.* 1969, Dyte 1970, Pinniger 1975, Khan 1981, Mondal 1986, 1988, Binns 1986, Saleem and Shakoori 1989, 1990, Rajendran 1990, Ali *et al.* 1991, Hussain 1995, 1996). No other control method has been established in Bangladesh so far except pesticides (Hussain 1996). The use of

synthetic pesticides grew enormously over the years as a result of which millions of tons of pesticides are being unused annually in Bangladesh (Ameen 1994). In Bangladesh, a total of 7.35 metric tons of pesticides were imported under 112 trade names that valued at Taka 106 crores during 1992. Since then, the use of pesticides increases in 1993 nearly 10,000 metric tones pesticides were sold to the farmers. More than 100 categories of pesticides have been registered and marketed by different companies in Bangladesh (Hussain 1996).

Insect Growth Regulators (IGRs)

The use of Insect Growth Regulators (IGRs) as an alternative method of pest control has received much attention recently. Growth regulators are of three kinds; juvenile hormones, ecdysome hormone and antichitin compounds. The spectrum of their biological activity ranges from ovicidal and larvicidal to lethal effects on pupae as well as adult (Mian and Mulla 1982a, b), and they are highly effective in causing morphological abnormalities (Williams and Amos 1974, Edwards 1976, Fajardo and Morallo-Rejesus 1980, Saxena and Mathur 1983, Eisa *et al.* 1984, Faragalla *et al.* 1985, Ishaaya *et al.* 1987, Parween 1996). At present in many countries various IGRs are being used to protect stored products from the attack of insect pests including *Tribolium* species. The application of IGRs for the supression of insect pest in stored commodities was first suggested by Thomas and Bhatnagar-Thomas (1968). In Bangladesh very few works on IGRs action on *Tribolium* have been done (Mondal *et al.* 1998, Mazid 2000).

Botanicals

The use of locally available plant materials to limit insect damage in stored foodstuffs is a common practice in traditional farm storage in developing countries (Hassanali *et al.* 1990, Dunkel *et al.* 1991, Poswal and Akpa 1991, Weaver *et al.* 1991, Bekele 1994, Talukder and Howse 1995). The use of pesticide of plant origin for the control of

agricultural pests has a long history but has assumed greater importance in recent years due to environmental deterioration and health hazards associated with the use of synthetic pesticides. Botanical pesticides exert a range of behavioural and physiological effects on the colonization, growth, survival and multiplication of insects. Natural plant products represent a very promising group of pesticide because of their apparent safety to mammals. In view of their environmental safety, these pesticides offer an attractive alternative to synthetic pesticides for use in IPM.

The impact of botanicals for the control of *Tribolium* has been illustrated by a number of studies. (Jacobson and Crosby 1967, Teotia and Tewari 1971, Agarwal *et al.* 1973, Saradamma *et al.* 1977, Tilak 1977, Schoonhoven 1978, Stenmark 1978, Cox 1981, Schmutterer *et al.* 1981, Prakash *et al.* 1982, Rajasekaran and Kumaraswami 1985).

Biological control

Khan and Mannan (1991) reported that biological control of pests have received much attention due to the obvious hazards produced by chemical pesticides. Virtually, natural enemies affect all insect populations to a greater or lesser extent. For many species, natural enemies are the primary regulating force in the dynamics of their populations (Hill 1983). A natural enemy usually reduces the subject insect population, the host or prey, by feeding on individuals, thereby promoting its own population at the expense of the population fed upon. Not only do this natural enemies help prevent some insects from attaining pest status, but they also play a role in reducing the damage potential of significant pests (Hill 1983). Haines (1984) has reviewed the current status of research on the role of parasites and predators in the management of storage pests.

Parasitoids have been used more frequently in biological control than any other kind of agent. Hymenoptera and Diptera are the most important parasitoid groups of insects among six parasitoid-including orders (Pedigo 1989). Parasitoids are often effective because of following: survival is usually good, only one (or fewer) host is required for complete development of a parasitoid, population can be sustained at low host levels

and most parasitoids have a narrow host range, often resulting in a good numerical response to host density.

Beside parasitoid characteristically, a predator is larger than its host, which it seizes and either devours or sucks dry of its body fluids rather quickly. Typically, a single predator consumes a number of prey in completing its development. Most predators are carnivorous in both their immature and mature stages and feed on the same kind of food in both stages. Predatory insects feed on all host stages—eggs, larval or nymphal, pupal and adult (Khan and Selman 1996). It is difficult to rank insect orders with regard to significance in predation because nearly every order has important species. However, with regard to diversity and significance of biological control, the Coleoptera, Neuroptera, Hymenoptera, Diptera and Hemiptera are outstanding (Pedigo 1989).

The use of insect parasitoids and predators to control stored-product insect pests has many advantages over traditional chemical controls. These natural enemies leave harmful chemical residues. Natural enemies released in a storage facility continue to reproduce as long as hosts are available and environmental conditions are suitable (Scholler and Flinn 2000). Unlike chemicals that need to wide area, natural enemies can be released at a single location and they will find and attack pests located deep inside crevices or with a grain mass. Parasitoids and predators that attack stored product pest are typically very small and have a short life cycle and a high reproductive capacity (Scholler and Flinn 2000). They can easily be removed from bulk grain using normal cleaning procedures before milling. In many ways the stored-product environment is favourable for biological control. Environmental conditions are generally favourable for natural enemies, and storage structure prevents these beneficial insects from leaving. Several reviews have been published on the use of insect parasitoids and predators to control stored-product insect pests (Burkholder 1981, Arbogast 1984, Haines 1984, Brower 1990, 1991, Brower *et al.* 1996, Nilakhe and Parker 1990, Burkholder and Faustini 1991, Scholler *et al.* 1997, Adler and Scholler 1998, Scholler and Flinn 2000).

Unlike chemical insecticides (Tyler *et al.* 1983) and insect pathogens (Fuxa 1993), pest insects have not yet developed resistance to parasitoids and predators (Hokkanen *et al.* 1995). It is likely that resistance to biological control agents will develop more slowly, or not at all, because the natural enemies are coevolving with their hosts and will tend to overcome host resistance.

Biological control shows promise in bulk storage, but may be more important in food processing facilities and warehouses (Cline *et al.* 1984, 1986, Prozell *et al.* 1995). Traditional insecticides are rather ineffective without a thorough cleaning of food processing and facilities. Small numbers of parasitoids could be released to seek out insect pests inside cracks and crevice. In the organic food processing industry and retail trade, effective control options are limited. Restrictions exist concerning the use of conventional insecticides. Therefore, the use of parasitoids and predators has become increasingly attractive to these industries. However, natural enemies will have to be used so that they do not contaminate the finished product. During the last 80 years, at least 900 studies about natural enemies of store product insect and mite pests were published (Scholler 1998). However, most of the studies were on the evolutionary ecology, toxicology, population ecology, and genetics of natural enemies. Comparatively few studies evaluated the effectiveness of natural enemies under practical condition of storage. In 1911, a parasitoid of the moth larvae *Venturia canescens* (Gravehorst) was observed in a flour mill in London. A sales circular was prepared that offered this parasitoid for sale to flour mills, and there were plans to export the species to Australia for sale. However, this first commercial venture failed when it was discovered that *V. canescens* already occurred in a number of flour mills in England and Australia (Froggatt 1912). Albrecht Hase was the first to systematically study the effectiveness of insect parasitoids in controlling stored-product insect pests. Limited field trials with the braconid wasp *Habrobracon* (= *Bracon*) *hebetor* (Say) to control the Mediterranean flour moth, *Ephestia kuehniella* Zeller date back to the 1920's (Hase 1922, 1925a, b). Hase also studied the biology of the parasitoids

Lariophagus distinguendus Foerster, *Trichogramma evanescens* Westwood and *V. canescense*.

The first recorded mass release of a parasitoid to control a stored-product pest occurred from 1942-1945, when 21, 798 *H. hebetor* were released in cacao-warehouses in Bahia, Brazil, infested with the tropical warehouse moth, *Cadra cautella* (Walker) (Silva 1947). The potential of the warehouse pirate bug, *Xylocoris flavipes* (Reuter) as a predator of stored-product pests was first evaluated by Jay *et al.* (1968). This predator was studied in subsequent years by Arbogast (1975, 1976). Classical biological control programs for stored-product insect pests started in 1991, when the histereid beetle *Teretriosoma nigrescens* Lewis was released in Togo, West Africa to control the larger grain borer, *Prostephanus truncatus* (Horn) (Markham *et al.* 1994). In the 1990's, research on biological control intensified and beneficial insects were evaluated in the laboratory and field.

Importance of Hymenoptera

The Hymenoptera as a whole is economically important upon humanity. The order is of particular importance as most of economically interesting species it contains are beneficial rather than pestilential species of Hymenoptera make food for us, pollinate our crops and destroy myriads of insect species.

Many ants and wasps are important predators of pest insects. Adult parasitoids engaging in host feeding after destroy large numbers of their hosts quite separately from those killed as a result of parasitism. Although a variety of biologists are known, species generally develop as parasitoids of a wide range of other insects. They are used as biological control agents. Repeated biological control success have proven that hymenopteran parasitoids can play a crucial role in the pest population regulation and, by extrapolation, suggest that they have an equal important role in the natural regulation of population of phytophagous insects (LaSalle and Gauld 1992, 1993).

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They provide commercial products such as honey, bees wax, etc. Only relatively small productions of the order are crop pests and forests pests. However, a few aculeates are notorious because they can inflict a painful sting.

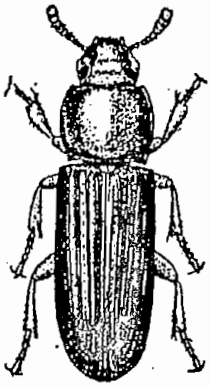
Importance of Hemiptera

Most of the hemipteran insects are phytophagous, a few are predators and parasites. The predatory hemipteran insects play a vital role for the control of pest insects. Predatory hemipterans may attack prey both as immatures and adults and consume more than one prey during their lifetime. Among Hemiptera some particularly important families are: Anthocoridae, Nabidae and Reduviidae (Pedigo 1996). Warehouse pirate bug, *Xylocoris flavipes* (Reuter) (Family Anthocoridae) is a predator of stored-product pests specially on *Tribolium* (Jay *et al.* 1968).

It is known that *Tribolium* is parasitized by two different parasitoids including, *Rhabdopyris zae* Waterston and *Holepyris sylvanidis* Brethes. One Predator, *X. flavipes*. *R. zae* and *H. sylvanidis* are the larval-pupal ectoparasitoids belonging to Bethyridae whereas *X. flavipes* is a predator under the order Hemiptera and family Anthocoridae. All these parasitoids and predator, more or less, are present in the natural condition and at certain time of the year they considerably check the pest population in the store.

Aim of the work

The aim of the present work deals with detailed biology of the parasitoids and predators of *T. castaneum* and *T. confusum*. The studies were courtship and mating behaviour, oviposition behaviour, morphology of immature stages, effect of foods on adult longevity, fecundity and sex ratio of *R. zae*, *H. sylvanidis* and *X. flavipes*.



Chapter 2

Review of literature

Review of literature

Works on the biology of Parasitoids/Predators of *Tribolium* are very few and reports dealing with these studies are comparatively less and scattered. However, an endeavour has been made to present here a brief review of the related literature available to date.

Bethylids are a species-rich and globally distributed family of aculeate wasps (Gauld and Bolton 1988). Adults range in size from about 1 to 10 mm. They parasitize the larvae, and sometimes the pupae, of Lepidoptera or Coleoptera. Uniquely among the parasitic aculeate families, a high proportion of bethylids are gregarious; all other families are largely solitary (Gauld and Bolton 1988). Eggs are laid on the exterior of the paralyzed host, often in very exact locations (Gordh and Hawkins 1981, Peter and David 1991). In many species, clutch size is known to be larger on larger hosts (Bridwell 1919, Kishitani 1961, Kuhne and Becker 1974, Gordh 1976, Gordh and Evans 1976, Gordh *et al.* 1983, Klein *et al.* 1991, Hardy *et al.* 1992, Luft 1993). Usually the host does not recover from paralysis. Some species have been observed transporting hosts from one location to another (Maneval 1930), possibly in an attempt to conceal them in a protective position. In a number of species, mothers defend the host and brood from conspecifics (Doutt 1973, Kuhne and Becker 1974, Hardy and Blackburn 1991, Petersen and Hardy 1996). Females may also destroy the eggs or larvae of any previous clutches they find (Geortzen and Doutt 1975, Legaspi *et al.* 1987, Hardy and Blackburn 1991). Such observations suggest that, in many cases, the offspring of only a single female complete development on a host. The larvae are immotile and hymenopteriform, develop in only a few days, and pupate near the host. The sex ratio of emerging offspring is usually female biased, more so for larger clutches, and the variance in sex ratio is often less than binomial (Green *et al.* 1982, Griffiths and Godfray 1988, Hardy 1992, Morgan and Cook 1994, Hardy and Cook 1995).

Developmental mortality is known in detail for eight bethylid species, with mean clutch sizes ranging from 2 to 15.6. In all eight species, more than one offspring normally

survives in gregarious broods; in seven of eight species, mortality is below 0.3. In addition, how mortality varies with clutch size is known for four species. In three species, mortality does not vary with clutch size, whereas in one species it decreases with clutch size. Furthermore, in one solitary species, a few instances of superparasitism are known, and more than one offspring is then able to complete development (Abraham *et al.* 1990). These data indicate that competition among bethylid larvae is generally scramble rather than contest. Direct observations of larval behaviour and development tend to support this view. Bethylid larvae do not change their position on the host after hatching and do not have fighting mandibles. Larval *Prosierola bicarinata* Brues however develop anterior protrusions that are inserted into the host and used to feed from parts of the host that are distant from the site of larval attachments (Doutt 1973).

Rhabdepyris zae Waterson is a parasitoid attacked different larval instars of the confused flour beetle, *T. confusum* and completed life cycle. Gahan (1930) also reported that this parasitoid reared in *T. confusum*. Ahmed and Islam (1988) for the first time recorded this parasitoid on *Tribolium* infested wheat and Bengal gram in the BCSIR Laboratories, Rajshahi, Bangladesh.

J J Davis sent four females and three males of a bethylid to the Bureau of Entomology for identification in 1929. He stated these bethylids were probably parasitoids of *T. confusum* and identified as *Rhabdepyris*. A female of the same species was received from Illinois State Laboratory of Natural History and reared from *T. confusum*. Five males of the same species have also been received from the Louisiana Experimental Station and reared from stored-corn insects at Baton Rouge by C O Hopkins in 1928. After a careful observation Dr James Waterston of the British Museum identified the specimens *Rhabdepyris zae* Waterston. It was originally described from a single female specimen taken at Liverpool England, in a shipment of maize from West Africa infested by *Calandra oryzae* Linnaeus, *T. castaneum* and *Laemophlaeus ferrugineus*

Stephens but never recorded from America. *R. zeae* parasitized several coleopteran pests of stored corn.

Ahmed and Islam (1988) published a brief biology of *R. zeae* on *T. confusum*. The parasitoid completed life cycle in 18-20 days under a constant temperature of $30 \pm 1^\circ\text{C}$. A mature larva formed silver white cocoon. Mating lasted for 2-3 minutes. The incubation, larval, pre-pupal and pupal periods were 1.5-1.8, 4-5, 3-4 and 9-10 days respectively. The parasitoid laid eggs on host larva between the fourth and fifth segments ventrally after carrying host larva into the tunnel of infested wheat grain. The fourth instar larvae were preferred for parasitization.

Holepyris sylvanidis (Brethes) was recorded for the first time as an coleopteran parasitoid by Brethes (1913). Brethes (1913) redesignated the parasitoids as *Holepyris sylvanidis* (Brethes). This parasitoid was found to parasitize *Sitophilus oryzae* (Linnaeus), *Oryzaephilus surinamensis* (Linnaeus), *L. ferrugineus* (Steph.), *T. castaneum* (Herbst), *T. confusum* Duval. It is distributed in Europe, Asia, USA, Barbados, Nicaragua, Trinidad, Brazil and Argentina.

Three species of bethylid wasps, *Prorops nasuta* Waterston, *Cephalonomia stephanoderis* Betren, and *C. hyalinipennis* Ashmead, attacked the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), by both predation and parasitism. All these bethylids were previously parasitized in the families Curculionidae, Bostrichidae and Bruchidae as alternate host reported Perez-Lachaud and Hardy (2001). High proportions of the parasitoids were fed upon and oviposited on. Offspring production per host was greater when *Sitophilus* were presented to females. Host feeding was very common in all the parasitoids. The lifetime fecundities of these parasitoids presented with these host species were, however, low (maximum 17 eggs) and preoviposition periods were generally long (29 days). Host feeding increased longevity.

Perez-Lachaud and Hardy (1999) studied the reproductive biology of *C. hyalinipennis*, a native parasitoid of the coffee berry borer, *Hypothenemus hampei* in Mexico. They found adult females lived upto 95 days (mean for mated females =57 days) and risk of death increased with age. Mating status, reproductive effort and female size influenced adult female longevity. Estimated mean lifetime fecundity was 88 eggs.

Ahmed and Khan (1994) recorded *C. waterstoni* Gahan parasitizing *C. pusillus* in larval stages. Mating in adults occurred within 2-3 hours of emergence. The preoviposition period ranged from 1.5 to 2.0 days. The average larval period occupied 5-6 days and the prepupal and pupal periods ranged from 1-1.5 and 7-8 days, respectively. The whole life cycle of *C. waterstoni* was completed in 15-17 days. The parasitoid larvae feed externally and devoured the body fluid of the host.

Biological characteristic of *Laelius pedatus* (Say), a braconid parasitoid of the Khapra beetle, *Trogoderma* described by Al-Kirshi (1998). Most egg-laying activity occurred at temperatures 28°C. The average number of eggs (62 ± 12.6) laid and deposited eggs developed to adults at extremely 90% at the same temperature. A mated female was able to deposit fertilized eggs for a maximum period of 14 days after copulation. The average lifetime of normally fed female lasted from 3 weeks at 35°C.

Howard *et al.* (1998) evaluated host-finding, host-recognition, and host-acceptance behaviour of *C. tarsalis* on saw-toothed grain beetle, *Oryzaephilus surinamensis* L. The authors reported some of sensory modalities that female *C. tarsalis* use to find, recognize and accept a host, and it provides an ethogram for the behaviour of the parasitoid from initial searching until she release the host in preparation for oviposition. Vision plays only a limited role in host-finding, and recognition. Chemical cues, primarily borne on the cuticle of the host and perceived through the wasp's antennae as well as movement by the host. Once contacted, are major host-recognition cues used by the parasitoid they found.

Reproductive behaviour of *Plastanoxus westwoodi* (Kieffer), an ectoparasite of *Cryptolestes pusillus* (Schon) is given by Ahmed and Khatun (1996). *P. westwoodi* mated within 24-36 hr of adult emergence which lasted for 35-45 seconds. The male took the initiative in mating and courtship. Before egg laying, the mated female parasitoids paralyzed the host larva by thrusting the ovipositor. The female laid eggs on the 2nd, 3rd and 4th instar or mature larva and pupae. Mature host larvae, however, were most preferred. The parasitoid sucked the body fluid of the host. The active feeding period lasted 1.5-2.0 days.

Hardy *et al.* (1998) described patterns of sex ratio, virginity and developmental mortality in some gregarious bethylid parasitoids. They reported *Goriozus nigrifemur* Ashmead and *G. legneri* Gordh are idiobiont gregarious ectoparasitoids of Lepidopteran larvae. The adult females attempt to exclude competing conspecifics from the vicinity of paralysed hosts. In *G. nigrifemur*, pairs of females fight for host possession and *G. lagneri* females respond aggressively to fine paint brushes gently pushed near to their hosts. Bethylids pupate around the remains of the host. Males typically eclose before females and may mate with their sisters either before these have left their cocoons or immediately afterwards. There is a lack of good evidence for the exact mating structure of bethylid species and some studies indicate a possibility of non-local mating.

Biology of *G. nephantidis*, a gregarious ecto-parasitoid of the larvae of *Opisina arenosella* Walker, a coconut defoliator in the Indian Sub-continent described by Cock and Perera (1987). Females *G. nephantidis* paralyse the host then lay a clutch of elongate 5-20 eggs on the host's integument. The developing wasp larvae feed as ectoparasitoids and pupating. Males generally emerge first after 5 days in a loose cocoon near the body of the host and mate with sisters either before these have left their cocoons or soon afterwards. Males mate with many females and one male *G.*

nephantidis is capable of inseminating at least 12 sisters under laboratory conditions. Unmated female produce male only. Female-biased sex ratio has been reported.

Nell *et al.* (1976) analyzed the oviposition behaviour of the females of *Encarsia formosa* Gah. towards larvae and pupae of host *Trialeurodes vaporariorum* (Westw.) in the laboratory. They showed that the parasite selected the third and fourth larval instars and pre-pupae for oviposition and selection were made with the aid of antennae and ovipositor. They assumed that host-feeding occurred at all stages but was the most frequent in second-instar larvae and pupae. Dhir (1977) also published detail oviposition behaviour of *D. vagabundus* Timb. The female of this species normally inserts its sharp and pointed ovipositor through the surface of the seed and places the egg on the body surface of growing larva of the bruchid within the seed. The author noted that the complete process of laying an egg required about 2-3 minutes when the larva was fully-grown and 5-7 minutes when the host was at its early stage of development. Dhir (1977) concluded that full-grown host larva was given distinct preference for oviposition.

van Lenteren *et al.* (1976) worked out the oviposition behaviour of the aphelinid parasite, *E. formosa* on host, *T. vaporariorum* on cucumber and tomato in the laboratory. For this study they showed that the parasite was able to detect parasitized and unparasitized hosts and avoided oviposition in the parasitized host. Rejection of the parasitized host took place after the female examined it with her antennae and sometimes with her ovipositor. They showed that when most of the hosts on a certain part of a leaf had been parasitized, the aphelinid left the site.

DeBach (1979) pointed out that the ovipositor lacks muscles except at its base but it is supplied with nerves extending its length to the tip which bears highly sensitive sense-organs that can discern by chemical stimuli whether a host is suitable or not and in fact whether it already contains a parasite. The ovipositor with the help of muscles

attached to its base can rotate back and forth like a drill and can flex in any given direction as it is used to explore either the surface or the interior of a host.

van Alphen (1980) studied the host-stage selection and found that both the eulophid parasites, *Tetrastichus asparagi* Crawford and *Tetrastichus* sp. oviposit in the eggs of *Crioceris asparagi* L. and *C. duodecimpunctata* L. containing advanced embryo or larvae. He found that both the species were able to discriminate between parasitized and unparasitized hosts after antennation or after probing with the ovipositor.

Fulton (1933) stated that the parasite *Habrocytus cerelelle* (Ashm.) inserts its ovipositor through the wall of the cell where the host larvae, *Sitotroga cerealella* (Oliver.) occupied. He observed that a viscid fluid oozed from the ovipositor after it was withdrawn from the host's body. The ovipositor was then reinserted into the oviposition puncture and the feeding tube completed. He noted that after this, the ovipositor was withdrawn slowly and carefully, then the female turned about and fed on the fluids coming out through the tube. Edwards (1954) indicates that the parasite, *N. vitripennis* lays eggs on host *Musca domestica* through the puparium with her ovipositor. The eggs are deposited on the body wall of the enclosed pupa. The female parasite then forms a feeding tube extending from the outside of the puparium to the pupa and sucks up fluid from the host-tissues. This food significantly increases the number of mature eggs in the female's ovaries. DeBach (1979) concluded that the ovipositor of some species is used to secrete and form a feeding tube through which the adult parasite sucks the body fluid in order to obtain protein for continued egg-production. A similar phenomenon was described by Wylie (1976) in case of *Euteromalus dubius* (Ashm.).

The effect of host-ages on rate of development of *N. vitripennis* was observed by Wylie in 1964 who recorded that the time was shorter on young pupae of less than 48 hr at $24.5 \pm 0.5^\circ\text{C}$ than on older pupae of *M. domestica*. This was primarily because of the intrinsic differences between young and old housefly pupae as hosts, the author

indicated. Subsequently he (1966), in another experiment, found that female parasites were larger in size when reared on fourth instar larvae, pre-pupa and pupae of the hosts. Okamoto (1971) recorded the developmental duration of the parasite, *A. calandrae* in relation to the different stages of *C. chinensis* under controlled conditions of temperature (30°C) and relative humidity (70%). He ascertained that the host is parasitized either at the third to fourth larval instars or at the pupal stage. The fourth larval instars were parasitized by all the wasps released, while the parasitization of the third larval instars and pupae were attained by a few of the released wasps. Later he found that when the parasite attacked the developmental stage of the host of 11-17 days after being oviposited, the mean developmental period of the parasite was about 14 days, while in the developmental stage of 7-10 days after being oviposited, the mean developmental duration was about 15 days. In another study Okamoto (1972) described the relationship between the developmental stage of the *C. chinensis* and *A. calandrae* and found that the pupal length of the parasite relatively varied depending on the size of the host.

Clausen (1939) noted that of the several factors that regulate the sex ratio in parasitic Hymenoptera, the two most important ones were the size and species of the host. DeBach (1964) enlisted many other factor that governs the sex ratio. These included (i) superparasitism that results in the production of male larvae which are smaller in size and need lesser nourishment; (ii) size of the host, which explains that bigger the parasitoids greater the possibilities of females and vice-versa; (iii) fertilized eggs produce females while unfertilized ones produce males; and (iv) temperature. Generally, low temperatures kill the sperms in the spermatheca of the female thus preventing fertilization.

Wylie (1976) recorded progressively lower percentage of female progeny in *E. dubius*, a parasite of cyclorrhaphous Diptera. Narasimham (1984) found in the eulophid parasite, *Aprostocetus hagenowii* (Ratz.) and *A. asthenogmus* (Wtstn.) of the cockroach

oothecae that the normal sex-ratio was biased in favour of females and was influenced by host-size and species, the proportion of females increasing with oothecae size.

The rate of development and size of the emerged adult aphelinid parasite, *E. tricolor* Forest from the glasshouse whitefly, *T. vaporariorum* were evaluated by Avilla and Copland (1987). They divided the developmental stages of the host into five nymphal instars (N1, N2, N3, N4 and pharate adult) and observed that the females developed faster when the egg was laid on N3 (18.0 days from egg) and slower on N1 (22.3 days). Females were bigger when developing from N1 and N3 than from N4 and pharate adult. Males always developed faster and were smaller than females, they observed.

Leius (1961a) observed greater reproductive activity and longevity of the females of *Itopectis conquistator* (Say) when they were supplied with carbohydrates and body fluids of their hosts. In a subsequent work he (1961b) found that the highest number of eggs was laid by the females of *Scambus buolianae* (Htg.) when they were fed upon the larvae of *Arachips cerasivoranus*. Wylie (1962) found the age of pupae of *M. domestica* affected the longevity and fecundity of *N. vitripennis* that fed on them. He observed longevity and fecundity of the female parasite as maximum when she fed on the pupae of less than 48hr old. Host feeding is generally regarded as a normal process in the pteromalidae as described by Clausen (1939). Legner and Gerling (1967) found that both the adults of *S. cameroni* Perkins and *N. vitripennis* required hosts for maximum longevity and fecundity. They also found that host-availability of young adult females apparently influenced the reproductivity and behaviour during their entire lifespan.

Moravskaya (1973) studied the effect of additional feeding on the fecundity and duration of life of imago of eupelmid egg parasite, *Anastatus disparis* and found that without imaginal feeding, the maximal lifespan of male and female were only 5 and 7 days respectively, but when fed on sugar syrup, the maximum lifespan of male and female were 16 and 32 days; and maximum number of eggs matured in one female was

9 but when fed on host-egg contents, the number of mature eggs attained was 16 and the maximum duration of life-span of female was 33 days. Patana (1979) observed that at 30°C more (227 ± 98.3) progeny of *Brachmeria ovata* (Say) were produced when fed on *Heliothis virescens* (F.). He observed that mean longevity ranged from 34.4 days at 35°C to 162.9 days at 20°C. The lifespan with honey as diet was also longer than with several other diets, viz., water, glucose, etc. of *E. formosa* as reported by van Lenteren *et al.* (1987). Timerak (1983) investigated the lifespan of adult *Bracon brevicornis* Westw. on various types of food or the absence of foods and found that when provided with artificial diets (sucrose, molasses, honey or tap water), the female parasite provided daily with a fresh larvae of *Sesamia critica* Led. lived longer than one kept permanently with the same larvae.

X. flavipes and several other anthocorid bugs of the subfamily Lyctocorinae frequently occur as predators in storage ecosystems (Arbogast 1979). The cosmopolitan species *Lyctocoris campestris* (F.), for example, is found in granaries as well as in a wide variety of other habitats; and *X. galactinus* (Fieber), a species found chiefly in habitats where the temperature is high, has been recorded from heating grain in Scotland and Canada. Also, the Australian species *X. queenslandicus* Gross has been reported from stored peanuts in Queensland, and we have collected *X. sordidus* (Reuter) and *Dufouriellus ater* (Dufour) from stored peanuts in Georgia. (*X. sordidus* occurs in Central and South America as well as in the United States, and *D. ater* is indigenous to Europe, North Africa, and the Near East, where it is found beneath the bark of various trees, but it has been collected a number of times from stored products in North America.). *Nidicola marginata* Harris and Drake, a species limited in distribution to California, Arizona, and northern Sonora, Mexico, has been collected from guano in bat caves, bags of cottonseed meal, the nest of a wood rat, decaying dates, and a culture of *Sitotroga cerealella* (Olivier).

Awadallah and Tawfik (1972) stated that mating of *X. flavipes* occurs on the day of adult emergence. The authors found there are five nymphal instars in the life of the

predator. The instars differ mainly in size, developmental of the wing pads, and color but color varies considerably within a given stage, particularly among 4th and 5th instars. The mean duration of nymphal instars. The mean duration of nymphal instars 1-4 is 2 days each at 30°C but the 5 instar is 3 days.

Arbogast (1975) observed that sex ratio of *X. flavipes* was 1:1. He further reported that each female laid an average of 41.6 eggs in a lifetime.

Arbogast *et al.* (1977) studied that at 30°C temperature virgin females lived larger than males, but mated females had a shorter lifespan than males. Starved nymphs and starved adults lived much longer at 20 than at 30° C. Twenty five of the females known to have mated oviposited. The total number of eggs laid by a single female ranged from 1 to 8, and the mean number (\pm SD) was 3.0 (\pm 2.0). Most eggs were laid the 2nd and 3rd days after mating. No developed or developing eggs remained in any females at the time of death.

Table 2. Species of insects on which *X. flavipes* is known to prey.

Order	Family	Species	Selected References
Coleoptera	Anobiidae	<i>Lasioderma serricornis</i> (F.)	LeCato G L and Davis R 1973. Preferences of the predator <i>Xylocoris flavipes</i> (Hemiptera: Anthocoridae) for species and instars of stored-product insects. <i>Fla. Ent.</i> 56 : 57.
	Bostrichidae	<i>Rhyzopertha dominica</i> (F.)	Jay E, Davis R and Brown S 1968. Studies on the predacious habits of <i>X. flavipes</i> (Reuter) (Hemiptera:Anthocoridae). <i>J.Ga. Ent. Soc.</i> 3 :126
	Curcujidae	<i>Oryzaephilus surinamensis</i> (L.)	Jay E, Davis R and Brown S 1968. Studies on the predacious habits of <i>X. flavipes</i> (Reuter) (Hemiptera: Anthocoridae). <i>J. Ga. Ent. Soc.</i> 3 :126 Arbogast R T 1976. Suppression of <i>Oryzaephilus surinamensis</i> (L.) (Coleoptera, Cucujidae) on shelled corn by the predator <i>Xylocoris flavipes</i> (Reuter) (Hemiptera: Anthocoridae). <i>J. Ga. Ent. Soc.</i> 11 : 67. LeCato G L, Collins J M and Arbogast R T 1977. Reduction of residual populations of stored-product insects by <i>Xylocoris flavipes</i> (Hemiptera: Anthocoridae). <i>J. Kans. Ent. Soc.</i> 50 : 84.

Order	Family	Species	Selected References
	Dermeestidae	<i>Attagenus megatoma</i> (F.)	LeCato G L. 1976. Predation by <i>Xylocoris flavipes</i> (Hem: Anthocoridae): Influence of stage, species and density of prey and of starvation and density of predator. <i>Entomophaga</i> 21: 217.
	Tenebrionidae	<i>Tribolium castaneum</i> (Herbst)	Jay E, Davis R and Brown S. 1968. Studies on the predacious habits of <i>X. flavipes</i> (Reuter) (Hemiptera:Anthocoridae). <i>J. Ga. Ent. Soc.</i> 3: 126 LeCato G L, Collins J M and Arbogast R T 1977. Reduction of residual populations of stored-product insects by <i>Xylocoris flavipes</i> (Hemiptera: Anthocoridae). <i>J. Kans. Ent. Soc.</i> 50: 84. Press J W, Flaherty B R and Arbogast R T 1975. Control of the red flour beetle, <i>Tribolium castaneum</i> in a warehouse by a predaceous bug, <i>Xylocoris flavipes</i> . <i>J. Ga. Ent. Soc.</i> 10: 76.
		<i>Tribolium confusum</i> Jacquelin duval	Jay E, Davis R and Brown S 1968. Studies on the predacious habits of <i>X. flavipes</i> (Reuter) (Hemiptera:Anthocoridae). <i>J. Ga. Ent. Soc.</i> 3: 126. Lecato G L 1975. Habitat-influencing predation by <i>Xylocoris flavipes</i> (Reuter) (Hemiptera: Anthocoridae). <i>Am. Midi. Nat.</i> 93:510.
Lepidoptera	Pyralidae	<i>Ephestia cautella</i> (Walker)	LeCato G L, Collins J M and Arbogast R T 1977. Reduction of residual populations of stored-product insects by <i>Xylocoris flavipes</i> (Hemiptera: Anthocoridae): <i>J. Kans. Ent. Soc.</i> 50: 84. Press J W, Flaherty B R and LeCato G L 1974. Interactions among <i>Tribolium castaneum</i> (Coleoptera: Tenebrionidae), <i>Cadra cautella</i> (Lepidoptera: Pyralidae), and <i>Xylocoris flavipes</i> (Hemiptera: Anthocoridae). <i>J. Ga. Ent. Soc.</i> 9: 101-103.
		<i>Plodia interpunctella</i> (Hubner)	Arbogast R T, Carthon M and Roberts J R, Jr 1971. Developmental stages of <i>Xylocoris flavipes</i> (Hemiptera: Anthocoridae), a predator of stored-product insects. <i>Ann. Ent. Soc. Am.</i> 64: 1131. LeCato G L and Davis R 1973. Preferences of the predator <i>Xylocoris flavipes</i> (Hemiptera: Anthocoridae) for species and instars of stored-product insects. <i>Fla. Ent.</i> 56: 57. Jay E, Davis R and Brown S. 1968. Studies on the predacious habits of <i>X. flavipes</i> (Reuter) (Hemiptera: Anthocoridae). <i>J. Ga. ent. Soc.</i> 3: 126.
Hymenoptera	Braconidae	<i>Bracon hebetor</i> Say	Press J W, Flaherty B R and LeCato G L. 1974. Interactions among <i>Plodia interpunctella</i> , <i>Bracon hebetor</i> , and <i>Xylocoris flavipes</i> . <i>Environ. Ent.</i> 3: 183.

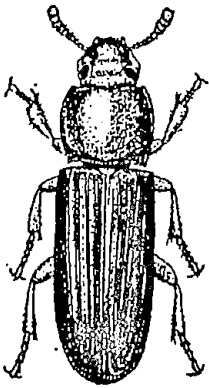
Ahmed *et al.* (1991) observed that the egg of *X. flavipes* is elongate-oval and blackish with yellow spots. The posterior end is broader than the anterior, which bears 3 evaginations. The egg ranged from 0.53-0.55 mm in length and 0.15-0.18 mm in diameter. The incubation period range from 4-5 days.

The author further described that *X. flavipes* passes through five nymphal stages of which 1st to 3rd instars nymphs may be termed as immature and 4th and 5th instars as mature nymphs. The 1st instar nymph is pale yellow, with reddish eyes, antennae and legs. Wings are not visible, but abdominal segments are distinct. The nymph measures on an average 0.57 mm in length, and 0.07 mm in head capsule width. The immature nymphs attacked the supplied prey at a slow rate. It is 1.20 mm in length and 0.14 mm in head width on an average. The antennae, eyes, wings and beak are prominent. The average duration of immature nymph ranged from 11.7 to 13.5 days in three generations.

Ahmed *et al.* (1991) observed that the mature (5th instar) nymph measures on an average of 1.4 mm in length and 0.21 mm in head width. Short dense setae on wings are distinct and the abdomen bear 2 bristles on each side. The mature nymphs are more predaceous than the immature nymphal stages.

The most sharply pronounced modifications are concentrated in the last moult from the final instar nymph to adult. The adult bug is blackish with yellow patches. The female is larger (2.0 mm) than the male (1.7 mm), while the female abdomen is six segmented and pointed, in male it is seven segmented and rounded. The adults of both the sexes predate on eggs and larvae.

The female mated with active male within 2 days after emergence. The oviposition period lasted for 15 days and a total of 87-95 eggs were laid ranging from 5-8 eggs per day. The percentage of egg hatching was nearly 63.22.



Chapter 3

Culture of *Tribolium* and the parasitoids and predator

Culture of the *Tribolium* and the parasitoids and predator

Collection of test *Tribolium* spp.

The adults of *T. castaneum* and *T. confusum* were collected from the stock culture maintained in IPM Laboratory, Institute of Biological Sciences, University of Rajshahi.

Culture of the red flour beetles

The beetles were sorted out to start a fresh culture. All equipment's were kept in an oven for sterilization, about six hours at 60°C (Khan and Selman 1981). Cultures were maintained in beakers containing food medium and pieces of crumpled filter papers placed inside the food medium for the easy movement of the beetles. The cultures were checked in regular interval, eggs and larvae were separated for proper growth. The beakers were covered with pieces of cloth tightly fixed with the help of rubber bands to avoid possible escape of the beetles. The beakers were kept in an incubator at 30° C ± 0.5°C without light and humidity control.

Preparation of the food medium

The whole wheat flour was used as the food medium for *T. castaneum* and *T. confusum*. The flour was sterilized at 60° C for four hours in an oven. A standard mixture of whole wheat flour with powdered dry yeast in a ratio of 19:1 (Park and Frank 1948, Park 1962, Zyromska-Rudzka 1966) was used as food medium throughout the experimental period. Food was not used until at least 15 days after sterilization to allow its moisture content to equilibrate with the environment.

Collection of eggs

About 500 beetles were placed in a 500 ml beaker containing food medium. The beaker was covered with a piece of cloth and kept in an incubator at $30^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. In regular interval the eggs were collected by sieving the food medium by two sieves of 500μ and 250μ aperture separating the adults and eggs respectively following the methods of Khan and Selman (1981). The eggs were then transferred to petri dishes (90 mm) and incubated at the same temperature.

Collection of newly hatched larvae

Larvae hatched out after 3-5 days in those conditions within an incubator that were then collected with a fine camel hairbrush and transferred to other food medium. This process was continued throughout the study period. Some were taken apart for tests on larvae and provided with a little quantity of food.

Determination of larval instars

Good (1936) reported that most larvae had six instars. The larval instars were determined by counting the number of exuviae (larval skin) deposited in the food medium according to Good (1933). Two days old larvae was considered as first instar larvae while second, third, fourth, fifth and sixth instar larvae were collected from larval culture on the fourth, seventh, tenth, thirteenth and sixteenth day from hatching respectively. After every three days the food medium was replaced by a fresh one to avoid conditioning by the larvae (Park 1935). Larval cultures were maintained in an incubator at $30^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ without light and humidity control.

Collection of newly formed adults

A huge number of flour beetles were thus reared to set a continuous supply of the newly formed adults. When sufficient adults produced in the subculture the adults were collected, separating them from the food medium. For this purpose some pieces of filter paper were kept inside the beaker on the food. The crawled adults on the paper were taken out with the help of forceps and the beetles were collected in a small beaker with the help of a camel hairbrush.

Precautions

All glasswares and sieves were sterilized at 180° C for about six hours in an oven and other materials washed with detergent. The experimental disks were cleaned every day.

Culture of parasitoids

The male and female parasitoids were collected from stored wheat and Bengal gram pest, *Tribolium* in the Integrated Pest Management Laboratory, Institute of Biological Sciences, Rajshahi University, Rajshahi. For studying the life cycle of the parasitoid, larvae of *T. castaneum* and *T. confusum* were reared on wheat flour kept in petri dishes (15 cm diam.) for three days. Then a large number of host larvae of different instars collected from the rearing medium were released. Some infested wheat kernels bearing minute holes were placed in the above petri dish to facilitate egg laying. Adult parasitoid after emergence were reared and allowed to oviposit in petri dishes containing host larvae. After egg laying the host larvae were dissected out from the seed-bores. The incubation period was carefully noted.

Immediately after hatching the parasitoid larvae were isolated in small petri dishes (5 cm diam.) and the larval, pre-pupal and pupal periods were recorded. Observations on egg laying and hatching were performed under binocular dissecting microscope. All the

measurements were taken with the help of ocular and stage micrometers. The drawing was made by camera Lucida (Magnification 5.5 and 5.10). All the experiments were conducted $30^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ in an incubator and based on observations.

Culture of predator

X. flavipes is also obtained from the Integrated Pest Management Laboratory, Institute of Biological Sciences, Rajshahi University, Rajshahi, rearing in stored wheat. The adults were collected from stored wheat infested by *Tribolium*. Female was kept in a petri dish (15cm diam.) containing twenty larvae and pupae of either *Tribolium* spp. to facilitate egg laying. The first instar nymphs after hatching were kept in six petri dishes (5 cm diam.) each having 10 individuals. The immature and mature nymphs were provided with early instar larvae of the prey. Regular observations at an interval of 24 hours were made to detect oviposition, 1st instar, immature and mature nymphal and adult stages. The experiments were conducted in an incubator at $30^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and 70% R. H.



Chapter 4

Courtship and mating behaviour

Courtship and mating behaviour

Mating is a normal and necessary process of the life cycle of each biparental parasitoid species (Stary 1970). In insect frequent mating is essential for maximum fecundity and fertility. Mating takes place immediately after emergence in most Hymenoptera. In arthropodous species a satisfactory sex ratio could best be maintained by habits which favour mating soon after emergence. This is aided in nature by the fact that males of most parasitic Hymenoptera have a shorter developmental period than the females and precede them to the adult stage by one or two days (DeBach 1964). Some attention has been paid to the mating habits of entomophagous Hymenoptera because a form of courtship is seen in the behaviour of the males of many species. In the Hymenoptera it is not uncommon to find that when a female is once fertilized, she will resist any further attentions of the males. Mating certainly has a remarkable effect in that it causes a significant behavioural change in the type of host selected and the manner of oviposition. Flanders (1946) indicates that the females of certain species are normally multinuptial, mating several times those of other species are uninuptial and refuse the subsequent males. Males exhibit distinct stages of excitation and display courtship behaviour before copulation. No overt courtship behaviour was observed in females. Multiple mating by males has been reported but females appear to mate only once. Works on the courtship and mating behaviour of *R. zae*, *H. sylvanidis* and hemipteran *X. flavipes* has not been conducted so far. Experiment was made to study the courtship and mating behaviour of *R. zae*, *H. sylvanidis* and *X. flavipes*.

Materials and methods

To observe the mating of the parasitoids cocoon of the parasitoids were kept individually in small vials (8.5cm) for emergence of adults. After emergence, a male and a female were placed in a foam screen-topped 4 ml vial containing 50% honey-

water in foam. The fifth stage nymphs of *X. flavipes* were reared in sterilized glass beakers (100 ml) for adult emergence. Mating was observed in direct eye observation.

In this way several mating sets were prepared and mating behaviour was observed under a stereoscopic binocular microscope. The mating behaviours, pre-mating and mating lengths were noted.

In order to observe the multiple mating or frequency mating of the male, mated female was removed soon after copulation and another freshly emerged virgin female were introduced. This procedure was continued and repeated until the male refused mating. During the experiment 50% diluted honey with water was served as and when necessary. The experiments were conducted at room temperature of $30 \pm 1^{\circ}\text{C}$ and 70 ± 0.05 relative humidity.

Results

Mating behaviour

The series of complex behaviours during mating may be divided into three phases, viz., phase of preparation, act of copulation and phase of post copulation.

Phase of preparation

To emerge, the adult parasitoids cut a circular hole on the upper surface of the cocoon. The male emerged one day earlier than the female. After one day of emergence the female was introduced into the vial and the mouth was quickly covered with screen. The males were extremely active and sought out of the females much of his time with a violent flapping of wings, chased the females and a quick agitated gait. While searching a male usually walks holding the flagellae of his antennae horizontal. The ventral surface of the antennal tips is occasionally lowered to the surface. The antennae are vibrated rapidly up and down with the antennal tips frequently contracting the surface of the host.

During this phase the female played passive role in comparison to the male. She oscillated her antennae slowly. She has bent her abdomen downwards, rubbed ovipositor sheaths by the pretarsi of the hind legs and during that period her wing seemed to be static, though she sometimes moved by walking or flies a little within the petri dish. She cleaned her mouthparts, ovipositor and legs in a very lazy motion. Males generally encountered the female while the latter was in comparatively static position. Approaching from the front or side of a female, a male depending on its position made contact with the female by his antennae placing face to face on diagonally. He then tried to mount on the female by a quick movement from front or side to rear. But the female who was not sexually responsive resisted the male in one or more ways. Normally, after first contact with the male she immediately displaced herself by taking a short flight or a run. The male moved from one place to another in the same manner rather than followed her. By this time he again encountered the female and exhibited the same phenomenon, which he did during previous contact. The non responsive female again might resist the male in the same way or she made kicking movement with her hind legs to dislodge the male. These sequences of events repeated for several times. In fact, the excitement of the female was a matter of gradual process. After 5-10 occasional contacts the dislodging or displacing tendency of female gradually decreased. On the other hand, the male in this phase (after 5-10 contacts) trailed forwards female. After repeated contacts the female at last gave positive signals for copulation. In this moment the antennae of the male contact the female, the male rapidly mounts on the female by jumping onto her dorsum and proceeds towards the head. Anterior wings of the male were held upward and downward direction by continuous rhythmic and fanning movement. This is a rapid, receptive movement in which the abdomen is elevated and the wings are fanned rapidly to produce an audible buzzing sound. The head and fore legs of the male are held above the vertex and catch the scape of the antenna of the female. The mesothoracic leg of the male overlaps the mesothoracic legs the female underneath, the head of the female pressing by side.

Metathoracic leg of male remains on the abdomen of the female by the side of the wing base. The head of the male is slightly down bent and the scapes of the male are held more or less straight and the wide spread flagella touch the antenna and face of the female. During courtship the head of the female is lowered so that the mouthparts contact the substratum. Thus, both the sexes entered into the next phase, i.e. in the act of copulation.

Like *R. zae* similar biological attributes were found in *H. sylvanidis*. Upon emergence the male and female parasitoids were sexually matured. Mating took place immediately after emergence.

The mating of *X. flavipes* usually occurs on the day of adult emergence. However, it appears from our observations that females are not sexually mature until 1 or 2 days latter. When females were held for 1 or 2 weeks after the final moult and then paired with males, partially developed eggs could be observed in cleared specimens within 1 day, and oviposition began within 2.

Act of copulation

In case of *R. zae* and *H. sylvanidis* after having mounted on the female the male struck the females antenna with its own. In the meantime, they maintained the opening and fanning of their wings and curved the tip of the abdomen under that of the female, the male tries to push its genitalia into the female gonopore. During the act of copulation, the females remain motionless. The male vibrates its antenna and forelegs holding the wings parallel with the body by a continuous rhythmic motion and move the wings in upward and downward directions for a while. At this stage, the head and forelegs of the male remain above the vertex of the female and touches the antenna of the female. The mesothoracic legs of the male overlap the pro- and mesoscutum of the female. The metathoracic legs of male are held on the proximal part of the abdomen of the female by her wing-base. The head of the male is partially lowered and the scapes of the male held straight; the flagella touch the antenna and face of the female. At that moment the

head of the female is bent down. After courtship, she draws her antenna forward and remains motions awaiting actual copulation. Courtship occurs about 2-5 times before commencement of actual copulation in both sexes.

Mating of the *X. flavipes* is via normal copulation instead of traumatic haemocoelic insemination. When male approaches a female and attempts to mount, he does so very fast, extending his aedeagus and jumping on top of the female. The mounting male immediately aligns his body longitudinally with the female, curves his abdomen down and inserts his aedeagus into the female.

Phase of post-copulation

After insemination the females of *R. zaeae* and *H. sylvanidis* awaked from its torpor and started to walk, thus forced the males to dislodge, within a few seconds the males exhibited the same pre-copulatory behaviours with the same mated females. But this time females refused to mate and always tried to keep her away from the males. After mating, the females of the parasitoid were busy to clean their body.

Length of pre-mating and copulation act

The pre-mating length was recorded as the time between emergence and one set of copulation act. The length of copulation act was recorded as the interval from insertion of the penis to the unclasping of the genitalia. The results are given in Table 3.

Table 3. Time spent of various mating activity of the parasitoids and predator (N=10).

Parasitoids and Predator	Duration (in sec.)			
	Pre mating		Act of copulation	
	Range	Mean \pm SE	Range	Mean \pm SE
<i>R. zaeae</i>	65-100	74.7 \pm 3.06	120-180	142 \pm 6.60
<i>H. sylvanidis</i>	50-70	60.0 \pm 2.20	80-100	85.4 \pm 1.89
<i>X. flavipes</i>	10-15	12.6 \pm 0.56	15-20	16.5 \pm 0.57

Multiple mating

Mated male of *R. zea* and *H. sylvanidis* were able to mate with several female up to 3-5 times at short intervals, when virgin females and honey were supplied to them. After first or second mating the males of the parasitoid species were found generally inactive for further mating. However, when honey was supplied to them, they regained their vitality and again start mating.

Mated female of *R. zea* and *H. sylvanidis* did not mate for the second time during whole life. If the female was not receptive she often kept her abdomen depressed when approached by a male.

X. flavipes shows multiple mating by females with the same or different males is common in this species.

Discussion

The adults were sexually matured on emergence and mating took place immediately after emergence in *R. zea* and *H. sylvanidis* a phenomenon that frequently occurs in many other bethylids. In the phase of preparation, the males play active role in comparison with the females (Mertins 1980, Gordh and Hawkins 1981, Gordh *et al.* 1983, Kapadia and Mittal 1986, Griffiths and Godfray 1988, Hardy and Mayhew 1998, Abraham *et al.* 1990, LeCato and Arbogast 1979). But the females receptivity are a major determinant of mating. Its influence is mainly on the duration of the pre-mating period and on the final success or failure of mating. Hence, this duration obviously differs from species to species. Courtship is brief and males exhibit elaborate specific courtship pattern, which includes searching, antennal contact, mount on the dorsum of the female, pressing antennae, movement of the flagellar part of antennae, rapid wing vibration, buzzing, dismount and copulation. All or most of the components: attraction, recognition, orientation, wing vibration, antennation, head movements, leg tapping, copulation and post copulatory grooming have been reported in different parasitic

wasps (Matthews 1975). He asserted that rapid wing vibration of male during courtship was probably in response to the female sex pheromone. Detailed courtship behaviour were also found in 19 species of bethylid parasitoids (Hardy and Mayhew 1998), 2 species of *Xylocoris* (Arbogast 1979). Coats (1976) observed that some males and females of *M. zaraptor* mate immediately after emergence. Some males exhibited pre copulatory behaviour although did not attempt insemination until they were a few hours older. Males continually pursued females because females walk until the male fully mounted. The males found the females more quickly (> 1 min. compared to > 3 min.) if the females were moving. Coats (1976) noted that movement by females played an important role in mating through (1) assuring visual recognition by males, since the multi-faced insect eye detects living organisms more readily by movement than by form (2) indicating non-receptivity by mated females during courtship. Ycargan and Braman (1986) studied the mating behaviour of the braconid parasite, *Diolcogaster facetosa* (Weed) parasitizing young larvae of green clove worm *Platynypena scraba* (F.) in the Eastern United States. In most cases the raising of the wings by males lasted less than 1 minute, but when within 1 cm. of the female, the males orient by bringing its abdomen down and forward to attempt copulation. Copulation required ca. 30-40s. During copulation, male parasitoid stayed behind the female and supported himself with his outstretched wings and the aedeagus was inserted at the anterior 3rd segment of the female abdomen. Similar mating behaviour were observed by Abraham *et al.* (1990), Griffiths and Godfray (1988), Cook (1993), Arbogast (1979).

Length of courtship and copulation has not been well reported in *R. zae*, *H. sylvanidis* and *X. flavipes*. However, a few authors reported the length of act of copulation for different bethylids viz., *C. stephanoderis* and *Prorops nasuta* 6.0 ± 0.7 seconds (Abraham *et al.* 1990), 2-6 seconds in *Goniozus legneri* (Gordh *et al.* 1983), and 38 seconds in *Laelius pedatus* (Mertins 1980). The length of act of copulation of another anthocorid bug *Lyctocoris campestris* is several seconds up to 35 minute (Parajulee and Phillips 1994). It has been observed in the present study that 74.7 ± 3.06 , 60.0 ± 2.20 ,

12.60±0.56 and 142.00±6.60, 85.40±1.89, 16.50±0.57 were spent respectively in pre mating and act of copulation by *R. zae*, *H. sylvanidis* and *X. flavipes* respectively. According to Collins and Grafius (1986) the mean duration of copulation of mymarid egg parasites *A. sodidatus* (Girault) was 56.7 ± 11.5 seconds. Males readily mated only once. Leonard and Ringo (1978) observed that chalcid females of *Brachymeria intermedia* (Nees) spent most of the time in searching and grooming. Some females stood still during the male courtship and lifted their abdomens and copulated. Copulation lasted 7-12 seconds.

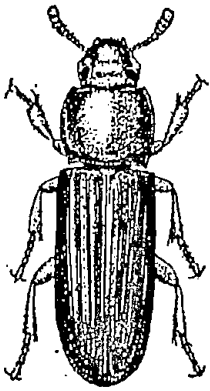
The above results show that the duration of copulation varies not only between different species but also some times it varies within the same species as in pre mating period. The reason behind these inconsistencies probably due to environmental factors during the experiments. So, the present variable results in the length of pre-mating and act of copulation in *R. zae*, *H. sylvanidis* and *X. flavipes* might also be due to above factors.

In the Hymenoptera, it is not uncommon to find that when a female is once mated successfully (fertilized), she will resist any further attempt of the male (Doutt 1964). This is true for bethylid parasitoids, because numerous reports of various authors also confirm that there is only a single mating in females during their lifetimes (Hardy 1992). Okeyo-Owuor *et al.* (1991) showed that both males and females mated repeatedly in *T. sesamiae*. Chien *et al.* (1991) reported that the males of *Tamarixia radiata* were capable of multiple mating with 93% of females. Carton *et al.* (1986) reported that the female pteromalid parasitoids of *Drosophila* (*D. dubius*, *P. vindemmiae*, *S. drosophilae* and *Toxoptera nigricola* Bouzek) are monandrous, while males are polygamous. In the present investigation a female of *R. zae* and *H. sylvanidis* were also found to mate only once in their life and hence, may be classified as belonging to the uninuptial group.

Contrary to females, multiple matings seem to be normal in males. The habits of multiple matings in males of *R. zae* and *H. sylvanidis* have not been reported earlier

clearly. It has been observed that single mated males of *R. zea* and *H. sylvanidis* were able to further mating about 3-6 times with different females.

The number of mating is apparently conditioned by the presence of food and temperature. In the present investigation, it has been observed that numbers of mating of males are directly correlated with the food. But the length of copulation act gradually decreases with the increase of successive mating of male, which was also reported by Hardy *et al.* (1999) and Arbogast (1979).



Chapter 5

Oviposition behaviour

Oviposition behaviour

The first relationship between the adult parasitoids/predator and host is through oviposition. The results of oviposition are the deposition of egg in the host. This begins the life of a parasitoid/predator. During oviposition, the parasitoids/predator shows conspicuous difference in her behaviour (Stary 1970). Ovipositional success is one of the important factors that result in effective parasitism. Successful parasitism requires completion of the following successive steps: habitat and host location, acceptance, suitability and regulation (Vinson 1976). The first three steps make up the host selection process. Vinson (1984) divided habitat location into two and acceptance into four stages. Thus altogether eight steps are involved in successful parasitism by parasitoids/predator. Habitat preference, potential host community location, examination, ovipositor probing, drilling, oviposition, and host suitability and regulation. Through this successive process, the host list becomes limited although many potential species are available in nature (Doutt 1964). The factors involved in successful parasitism have been more frequently studied. Ovipositional success is one of the important factors that result in effective parasitism. Oviposition behaviour does not always imply successful oviposition (LeMasuier 1990). So the study of oviposition is essential to know the biology of parasitoids/predator. The oviposition behaviour of parasitoids/predator of *Tribolium* has not been conducted elaborately. This led to the present experiments.

Materials and methods

Mated females of the parasitoids were collected from the previous mating sets and each was introduced in separate petri dishes (8.5 cm diam.) containing larvae of *Tribolium* for oviposition. A single female parasitoid was introduced into the petri dish. The female parasitoids used in the study were reared on *Tribolium* obtained from laboratory cultures.

Three sets of observation were made. In the first sets, parasitoids were used to study the behavioural sequence during oviposition. For these, 2-3 days old equal sized adult females, which had been fed with 50% honey solution, were allowed to mate and kept with 7-14 days old *Tribolium*. In the second set, parasitoids were selected for parasitizing *Tribolium* to observe the influence of oviposition. For these, equal aged mated and fed females, which had been deprived of host, was used. The parasitoids were then transferred to new host *Tribolium* after each oviposition. If the wasp showed no response to the *Tribolium* after 30 minutes the observation was discontinued. In the third set, equal aged, sized, fed and mated females were allowed to oviposit in the host *Tribolium* to count frequency of successful egg deposition.

The observations were carried out under a stereoscopic binocular microscope and all the events were noted. The pre-oviposition period and length of act of oviposition were also noted by stopwatch. The above experiments were conducted at $30\pm 0.5^{\circ}\text{C}$.

Results

Eighteen discrete ovipositional behavioural categories were recorded in the interaction of *R. zea* and *H. sylvanidis* females and their hosts *Tribolium*. These categories and their description are described bellows.

Search: Movement of parasitoid in response to its environment apart from direct interaction with host. Consists of walking, running, antennae waving or touching surroundings. Usually ultimately leads to host location by either random processes or directed ones.

Move away: Any movement by the wasp away from the host after the host has been contacted. Consists of walking or running, moving away as a defensive behavior, being pushed away or thrown off by host.

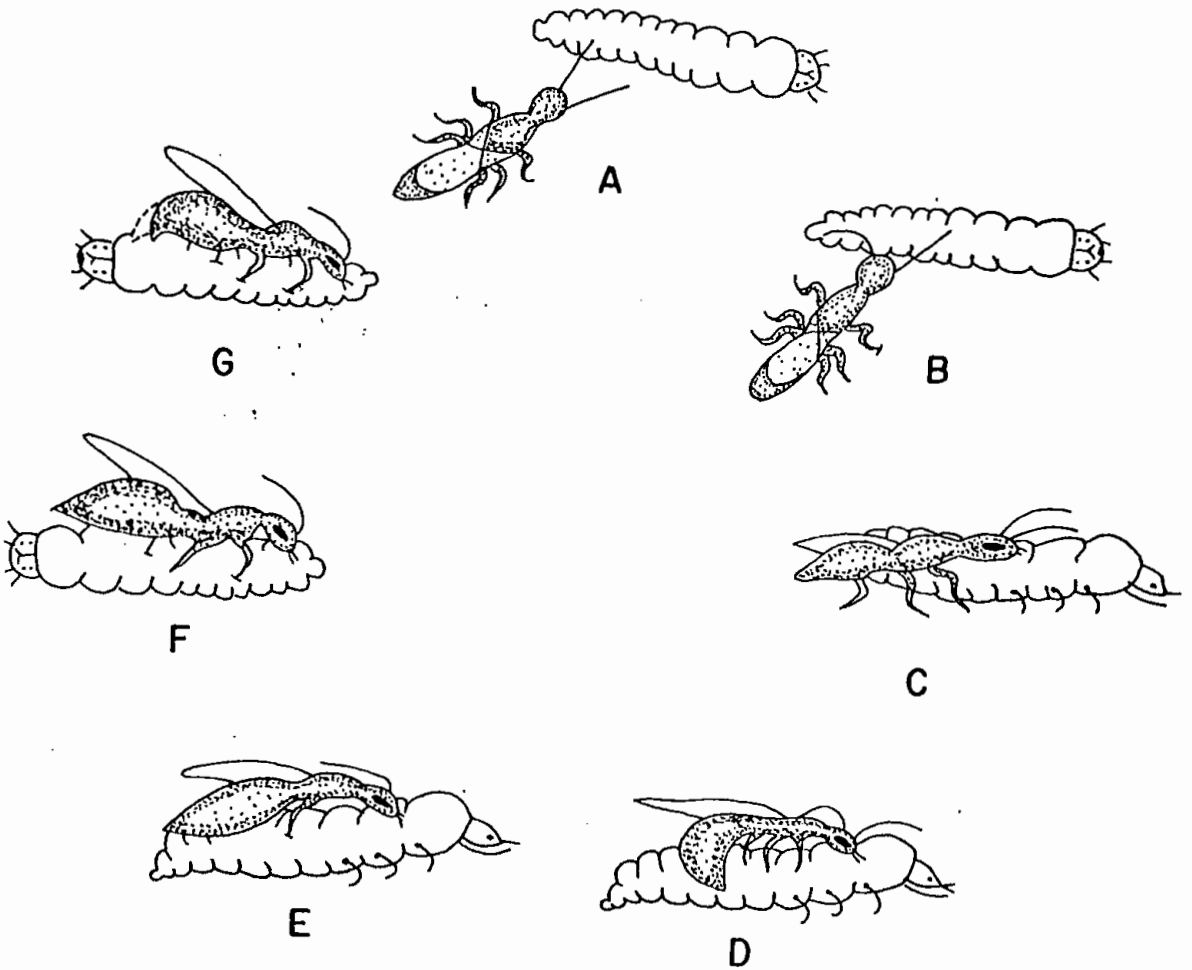


Figure 1. Diagrammatic sketches of 7 behavioural interactions between *R. zea* and *T. castaneum* larvae:

- | | | | |
|-------------------|------------------|---------------|----------|
| A. Antennal touch | B. Antennal side | C. Head/Mouth | D. Sting |
| E. Catatonic | F. Chew | G. Rub. | |

Bite: Parasitoid grabs host with its mandibles and successfully pierces the host integument preparatory to stinging.

Antennal Tip: Touching host with 1 or both tips of the antennae, either 1 time or several times in a row, uninterrupted by any other behavior.

Antennal side: Same as antennal tip except the sides of the antennae are used for touching.

Head/Mouth: Moving head or mouth close to host and touching without biting.

Clim on: Climbing up on host larvae in either a parallel or perpendicular orientation.

Sting: Curling of abdomen around host and inserting sting into host.

Turn: Change in body direction of wasp by at least 90 degrees in response to host movement.

Pause: A momentary cessation of all obvious behavior except for antennal waving.

Attempted Bite: Parasitoid attempts to grab larvae with its mandibles, but fails to do so.

Groom: Self-grooming by the parasitoids.

Catatonic: When the parasitoid stases after she has either attempted to or has stung the host. The wasp continues to hold on with its mandibles, but ceases all other movement, including antennal waving. This state continues until the host's movements have slowed to almost complete cessation, or until after several seconds of no change in the host's movement, when the parasitoid begins to move again.

Chew: When the parasitoid has its mandibles embedded in a paralysed host and appears to be extracting fluids from the host. There is little movement except for slight rhythmic movement of the parasitoid's head and mandibles, with an occasional pumping motion of her abdomen.

Pull: When a parasitoid grabs the host larvae with her mandibles and pulls (or pushes) it on the substrate.

Rub: Parasitoid rubs host larvae with the tip of her abdomen.

Touch: Includes both antennal tip and antennal side touching.

Rest: Parasitoid tucks head downward against substratum and ceases all movement except for an occasional abdominal pumping motion or twitch. This lasts for at least 30 seconds.

The diagrammatic drawings detailing the behavioural interactions of *R. zea* females with their normal host, *Tribolium*, is presented in Figure 1. The most typical behavioral sequence involved searching for the host, followed by antennal contact, climbing onto the host, biting it, and stinging it. This was followed by 15-30 seconds of catatonic immobility until the host's movement ceased, after which the wasp pulled the host on the substrate for a short distance, climbed back onto it, rubbed and chewed it. The wasp then moved away, and began grooming and resting.

The oviposition behaviours of *R. zea*, *H. sylvanidis* and *X. flavipes* are more or less similar. After mating *X. flavipes* normally laid eggs flat petri dish. At that time of egg laying the female gets a move on the petri dish.

Discussion

The general scheme of oviposition posture of *R. zea* and *H. sylvanidis* and *X. flavipes* is almost similar to the general scheme of posture shown by other hymenopteran parasitoids and hemipteran predators. However, differences exist in their actions or behaviour such as, holding of head, antennae, wings, legs and abdominal curvatures during the act of oviposition in different species (Edwards 1954, Doult 1964, Corrigan *et al.* 1991, Gardenghii *et al.* 1991, van Alphen and Vet 1986, Vet *et al.* 1995, van Alphen and Jervis 1996, Quicke 1997).

Edwards (1954) reported five distinct phases of oviposition behaviours in pteromalid *Nasonia vitriperrines* viz. finding the host area, finding a fly puparium, drumming and drilling response and oviposition and feeding response. The antennae and ovipositor help to locate the suitable place for oviposition van Lenteren *et al.* (1976) has also described it. Doult (1964) mentioned five distinct phases of oviposition behaviour, which included: drumming, tapping, drilling, ovipositing and with drawing. Gerling

and Legner (1968) argued that the female *Spalangia* drummed the host puparium, tapped it with the tip of her abdomen and finally drilled into it at a selected point. Corrigan *et al.* (1991) mentioned that fifteen compounds of ovipositional behaviours were identified in females of the eulophid *Edovum puttlerii*, Gardenghii *et al.* (1991) observed in the same species (*E. puttleri*) are three patterns were observed: drilling, host feeding and oviposition. Howard *et al.* (1998) described eighteen distinct phases of oviposition behaviour in different bethylidae which are similar correspondence in the present study. However, differences were noted in both duration of the sequences of behaviours and other related phenomena. Stand and Vinson (1983) investigated the host acceptance behaviour of *T. heliothidis* was broken into discrete steps: host encounter, drumming, and adoption of drilling posture, probing, drilling, oviposition and marking. Works of Flanders (1939) suggest that females of some species of hymenopterous parasites control the sex of their progeny by controlling fertilization immediately before oviposition. He noted that fertilization of eggs depended largely on the suitability of the host, those that are smaller or are less nutritious, tend to receive unfertilized eggs that produce males, van Alphen and Thunnissen (1983) observed similar phenomenon in the parasite, *Pachycrepoideus vindemmiae* Rondani. During the present investigation it was observed that unmated female only produce male. In the present study *R. zae* and *H. sylvanidis* exhibited sequence of oviposition behaviour similar to those described by Hardy (1994), Perez-Lachaud and Hardy (2001). However, differences were noted in both duration of the sequences of behaviours and other related phenomena.

Oviposition may be accompanied by host feeding. Host feeding and host predatism by the adult females is known to be fairly common among the parasitic Hymenoptera (DeBach 1943). The adult females of different bethylids parasitized several hosts in their lifetime. Al-Kirshi (1998) *Laelius pedatus* (Say) produced fertilized eggs for a maximum period of 14 days. Perez-Lachaud and Hardy (2001) reported both host feeding and the physical preference of the coffee berry borer, *Hypothenemus hampei* (Ferrari) late larvae, prepupae, or pupae are required for oogenesis in *C. stephanoderis*.

They found *C. hyalinipennis* when offered individual host, *H. hampei*, the bethylid females generally practice concurrent host feeding: individual hosts were often used for both feeding and oviposition. Females feed on about half of the hosts they attacked and oviposited on the other half and the proportion of hosts used for oviposition increased as females aged. The females appear to make feeding/oviposition decisions on the basis of their own age and host size, these decisions may also be influenced by the number of hosts currently available: if only one host presented, females have to use it for both feeding and oviposition in order to reproduce. Similar observations observed by (Abraham *et al.* 1990).

The literature on parasitoid, host-finding, recognition on acceptance is extensive and has been well-documented (van Alphen and Vet 1986, Vet *et al.* 1995, van Alphen and Jervis 1996, Quicke 1997). Although stored-product insects are found in a diversity of situations, generally hosts located in stored grain commodities are in a darkened environment with little air movement and only modest daily changes in temperature or relative humidity. Furthermore, the commodities are frequently present as extremely large masses (often several tons) and the host insects are present in relatively low levels (the economic threshold is commonly given as 1 insect per kilogram of host insect) (Hagstrum and Flinn 1995). The problems presented for the parasitoids in finding their hosts and therefore substantial and possibly involve different strategies from those found for the parasitoids of other groups.

R. zea females have well-developed eyes and might be expected to use vision to locate their hosts in the stored commodities, at least when the hosts are near the surface of the stored commodity where some light might be present.

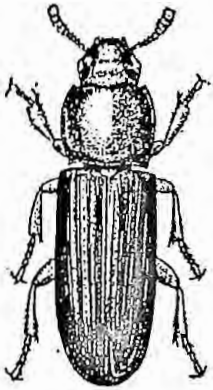
Although *Tribolium* larvae may not be leaving a directional chemical trail that *R. zea* females use, it is clear that the parasitoid is using chemical cues perceived by their antennae to locate and recognize the host larvac. Upon locating the beetle, the parasitoid briefly antennates it with outstretched antennae and proceeds to grab hold of it and sting it clearly,

the parasitoid's antennae are necessary, whereas its maxillary and labial palps do not seem to be of importance, because removing the palps does not hinder successful attack. Cuticular-borne chemical cues probably provide the primary signal that the correct host has been found, but the wasp also requires that the beetle respond to its attack by moving.

The ovipositional behaviour displayed by *R. zae* females after they contact the host is shown in Figure 1. The sequence of climbing onto the host, biting it and stinging all occurred very rapidly. If the parasitoid was successful at embedding her mandibles into the cuticle of the host (biting), then she was inevitably successful in stinging and paralyzing the host. Occasionally >1 sting was required to subdue the host. The parasitoid pulls the host to a suitable location to hide it; then the parasitoid climbs back up on the host, rubs the larva all over with tip of her abdomen, and then apparently host feeds (chew). Host feeding is a common strategy among parasitoids (Heimpel and Collier 1996) and is thought to be important for both somatic maintenance and reproductive development. In addition, Howard *et al.* (1998) observed that the cuticular hydrocarbon profiles of *R. zae* were dependent on whether the female had host-fed or not. The rubbing behavior of *R. zae* is very similar to a behavior displayed by *C. tarsalis* and *C. waterstoni* females while they are preparing their paralyzed host for oviposition. Because *R. zae* does not oviposit until long after this rubbing behavior, it is possible that she is leaving some sort of a semiochemical mark of unknown function. After host feeding, the parasitoid leaves the host, grooms herself, and then rests.

Although *R. zae* and *H. sylvanidis* females fed and oviposited on *Tribolium*, there was an extended preoviposition period (14-20 days) in the present observation. Perez-Lachaud and Hardy (2001) described alternate hosts of some bethylidae parasitoids are found preoviposition period 16-19 days, females provided one coffee berries pupae/day have larger preoviposition periods than those with 10 hosts/day and this probably accounts for the long preoviposition period and low fecundities (Lauziere *et al.* 1999). The preoviposition period of *Prorops nasuta* Waterston is high but within the range (8-39 days) (Abraham *et al.* 1990).

Arbogast (1979) observed that adult lifespan and oviposition period of *X. flavipes* were longest at low temperatures and intermediate humidities. Both became progressively shorter as temperature increased, and the maxima shifted to progressively higher humidities. The maximum number of eggs was laid between 25 and 28°C. Humidity had little effect on oviposition below about 60% R.H., but the number of eggs laid diminished with increasing humidity above this value. The rate of population growth was greatest within the zone bounded by 29 and 31°C and 60 and 70% R.H. It declined rapidly with increasing temperature above 33°C, especially at low or high humidities, and also declined, but more gradually, with decreasing temperature. He also observed when females were held for 1 or 2 weeks after the final molt and then paired with males, partially developed eggs could be observed in cleared specimens within 1 day, and oviposition began within 2; when they were paired as soon as they emerged, oviposition did not begin for 3 or 4 days. Arbogast *et al.* (1983) also noted that another anthocorid bug of *X. sordidus* (Reuter) species prefers to oviposit in a soft substrate such as pith. A related species, *X. galantinus* (Fieber), also appears to prefer hot, moist conditions where it may be primarily a predator of house fly larvae, *M. domestica* (Hall 1951). The preoviposition period of female *Lyctocoris campestris* that has been paired with a male since emergence ranges from 5-15 days when *P. interpunctella* is the prey (Parajulee and Phillips 1993). They described a strong correlation between the length of the oviposition period and total number of eggs laid per female has been found irrespective of the prey species. Parajulee and Phillips (1994) observed that *L. campestris* has successful oviposition and subsequent embryonic development have been achieved only on a moist substrate, preferably a stack of three filter papers saturated with the water. They also observed the possible utilisation of an oviposition substrate that may not require water-saturation. Female spend an average of 82.2 (± 2.4) seconds for each oviposition. This time period includes abdomen bending, touching the substrate with the ovipositor, inserting the egg into the substrate, and elevating the abdomen back to the beginning position.



Chapter 6

Morphology of immature stages

Morphology of immature stages

The study of immature stages is important in any biological investigation of parasitoids since it generally offers the best clues to the nature and kind of parasitoid (Hagen 1964).

As in other protelien parasites, only the immature stages of bethylids, eulophids, eulophids, eupelmids and evaniidae are parasitic, whereas, the adults are free living. The immature stages *viz.*, eggs, larvae, pre-pupal, pupae differ markedly from adult in structure, behaviour, food habit and habitat. All the pre-adult stages of the parasitoid are passed well protected within the larval instar. The eggs of the parasitoids are mono embryonic type and subsequently matured through different larval instars. The mature larva pupates inside the cocoon from which the adult emerges.

Materials and methods

The male and female parasitoids and predator were collected from stored wheat infested *T. castaneum* and *T. confusum* in the Integrated Pest Management Laboratories, Institute of Biological Sciences, Rajshahi University. For studying the life cycle of the parasitoids, larvae of *T. castaneum* and *T. confusum* were reared on wheat flours kept in petri dishes (15 cm diam.) for three days. Then a large number of host larvae of different instars collected from the rearing medium (11 cm diam.) where some mated female parasitoids were released. Some infested wheat kernels bearing minute holes were placed in the above petri dish to facilitate egg laying. Adult parasitoid after emergence were reared and allowed to oviposit in petri dishes containing host larvae. After egg laying the host larvae were dissected out from the seed-bores. The incubation period was carefully noted. Immediately after hatching, the parasitoid larvae were isolated in small petri dishes (5 cm diam.).

The parasitoid larva crawls over the host body and finally select a suitable position for feeding. The larva becomes fixed at a suitable position after 20-25 hours. The larva continues to suck the body fluid of the host on the second day for its development. At this stage the mouthparts of the parasitoids are highly on the host's body and moves their head a little from one side to the other.

The larva remains attached to the host's body and the mouthparts remain in close contact of the upper surface of the host body on the third day and become larger in size.

On the final day, the parasitoid larva completely encircles the host larva. The mouthparts penetrate more deeply in the host-body to get more fluids for its development. After becoming full-grown the larva changes to pre-pupal or finally pupal stags. Due to constant sucking, the host body shrinks to become smaller in size and finally it dies. Subsequently the larval, pre-pupal and pupal periods were recorded. Data were obtained from at least 3 sets observations.

The newly deposited eggs were studied. The tracheal system and body segmentation were studied in fresh larvae, because at that condition the system is the best observable (DeBach 1964, Corbet and Rotheram 1965). The larvae were washed in a few drops of 10% saline solution on a grooved slide before observation. Through observation of the parasitoid eggs, larva, pre-pupa, pupa were made after preparation of their permanent slides, they were preserved in Kahles fluid for 10h, transferred to 70% alcohol, heated with 10% KOH for a few minutes and then passed through water, acetic acid, dilute acid fuchsin stain, carbol-xylot and finally mounted in DPX. The larvae and pupae were placed laterally on the slides, while the mouthparts of different aged larvae were dissected out and placed dorsally and measurements were taken. All the measurements were taken with the help of ocular and stage micrometers. The drawings were made by camera Lucida (Magnification 5.5 and 5.10). All the experiments were run $30 \pm 0.5^\circ\text{C}$ in an incubator and based on twenty observations.

Results and observations

R. zae and *H. sylvanidis*

The parasitoids are generally holometabolous, i.e. they have a complete metamorphosis and pass through the eggs, larval instars, pre-pupal, pupal and adult stages. The immature stages of *R. zae* and *H. sylvanidis* are parasitic whereas the adult stages are freeliving. The immature stages differ markedly from adult in structure, behaviour, food habit, food requirement and habitat. The details of egg, larval, pre-pupal and pupal stages of this parasitoid are as follows:

Egg

After mating, the female *R. zae* partially paralyzes the host larva attacking at its abdominal region and bites the leg at tibial and tarsal joints. Then the parasitoid carries the host larva into the tunnels of infected wheat kernel for egg laying. The egg is about 0.51 mm in length and is glued to its length along the middle of the 4th and 5th segments ventrally. Sometimes *R. zae* egg is fixed along the side of the body of the host. The eggs of both the parasitoids are monoembryonic type and subsequently matures through different larval instars. The egg is elongated, slightly broader and blunter at the head end which, however, points towards the posterior extremity of its host. Freshly deposited egg is whitish, gradually changing into pale yellowish at the central portion before hatching. The incubation period ranges from 1.5-1.8 days.

After mating the female *H. sylvanidis* deposit eggs on the external surface of the host of *T. castaneum* and *T. confusum*. The egg is elongated, smooth and dirty white in appearance. The external envelope or chorion of the egg is chitinized, transparent and evenly surrounded the egg. The incubation period ranges from 1.5-2.0 days.

Both the parasitoids possess ovaries in which eggs are matured throughout all or most of adult life. The parasitoids are synovigenic, the female laid single egg in a single host. Any eggs present are not likely to be well developed and will require the female to

host-feed to mature them (Jervis and Kidd 1986, van Lenteren *et al.* 1987, Collier 1995). If prevented from host feeding, then there is ample precedent for the female reabsorbing the developing egg and using the nutrients for either her own maintenance or the development of future eggs (Heimpel and Collier 1996).

Duration and measurement of the eggs, larva, pre-pupa, pupa and the adults of parasitoids and predator are presented in Appendix Table 1, 2, 3, 4, 5, 6 and Table 4, 5, 6.

Larval instars

The chorion ruptures on the dorsal surface to allow sufficient space for the escape of the larva. Larval movements possibly help to recede out of the chorion.

There were three larval instars in the life of *R. zae* and *H. sylvanidis*.

First instar

The newly hatched larva of *R. zae* measures 0.42 ± 0.02 mm in length and 0.15 ± 0.01 mm in head capsule width. The first day larva, crawling out to segment 6, commences to feed on that segment. It is successful in penetrating or drawing nourishment through the tough integument of the beetle larva. The host larva then continuously moves its head region and legs to get rid of the parasitoid larva. The host larva remains actively alive upto the 2nd day age of the parasitoid infestation, from then it enters into the moribund state. The *H. sylvanidis* newly hatched larvae are smooth, translucent and dirty white, except the middle region which appears rather opaque. The larval body of both the parasitoids consists of 13 segments behind the head segmentation of body is externally prominent at the marginal areas only, but at the middle part segmentation is not well defined. The head capsule bears unsegmented horn-like antennae. The mandibles (Figures 2, 3) were observed at the base of the first segment. The mandible of first instar larva of *R. zae* has an average length of 0.04 mm (range: 0.03-0.04). The mandible of first instar larva of *H. sylvanidis* has an average length of 0.03 mm (range: 0.03-0.04). The mandibles are transparent with sharply pointed tooth in both *R. zae* and *H. sylvanidis*.

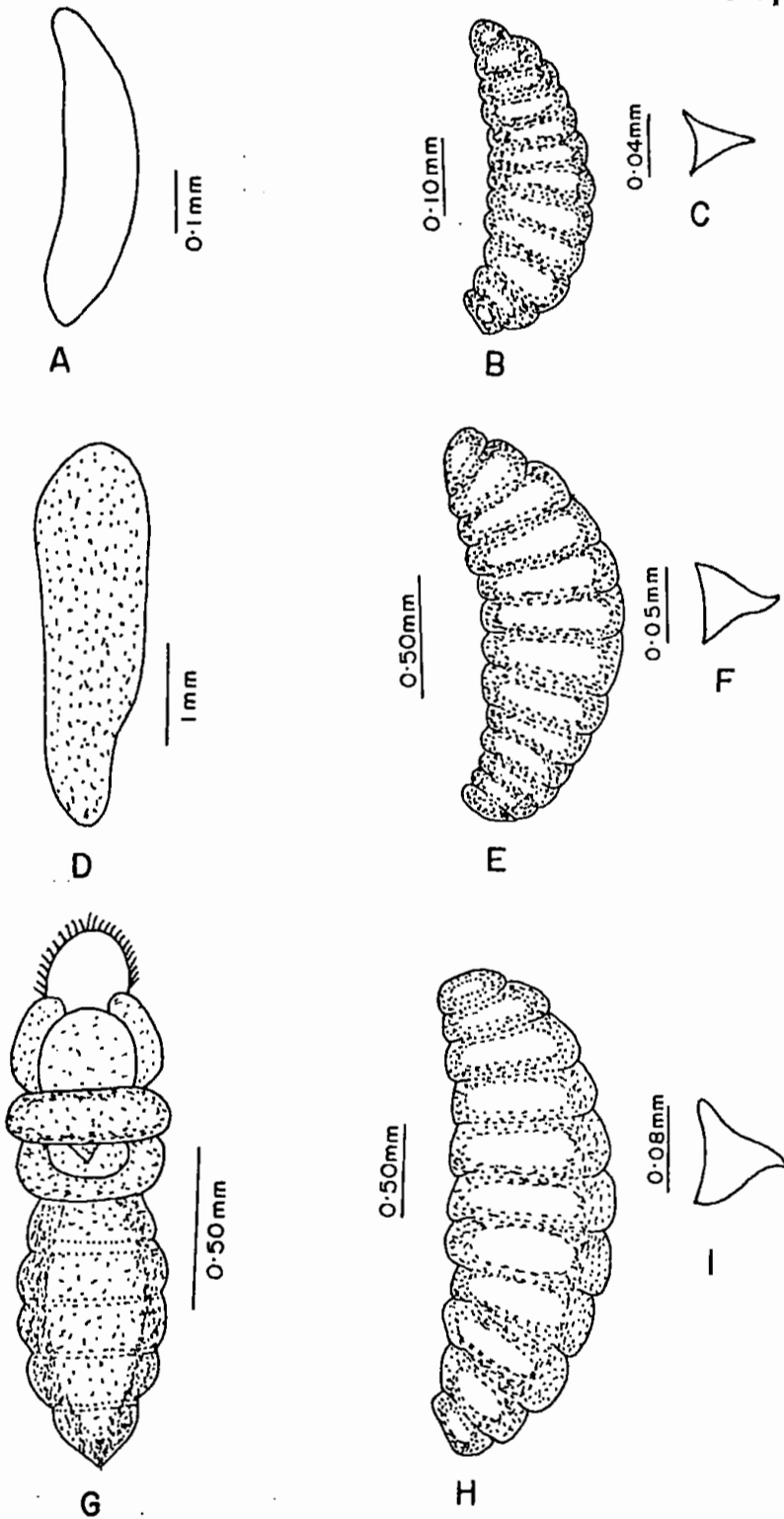


Figure 2. Development stages of *R. zae* :

- | | | |
|---------------------|---------------------------------|--|
| A. Freshly laid egg | B. 1 st instar larva | C. Mandible of 1 st instar larva |
| D. Cocoon | E. 2 nd instar larva | F. Mandible of 2 nd instar |
| G. Pupa | H. 3 rd instar larva | I. Mandible of 3 rd instar larva. |

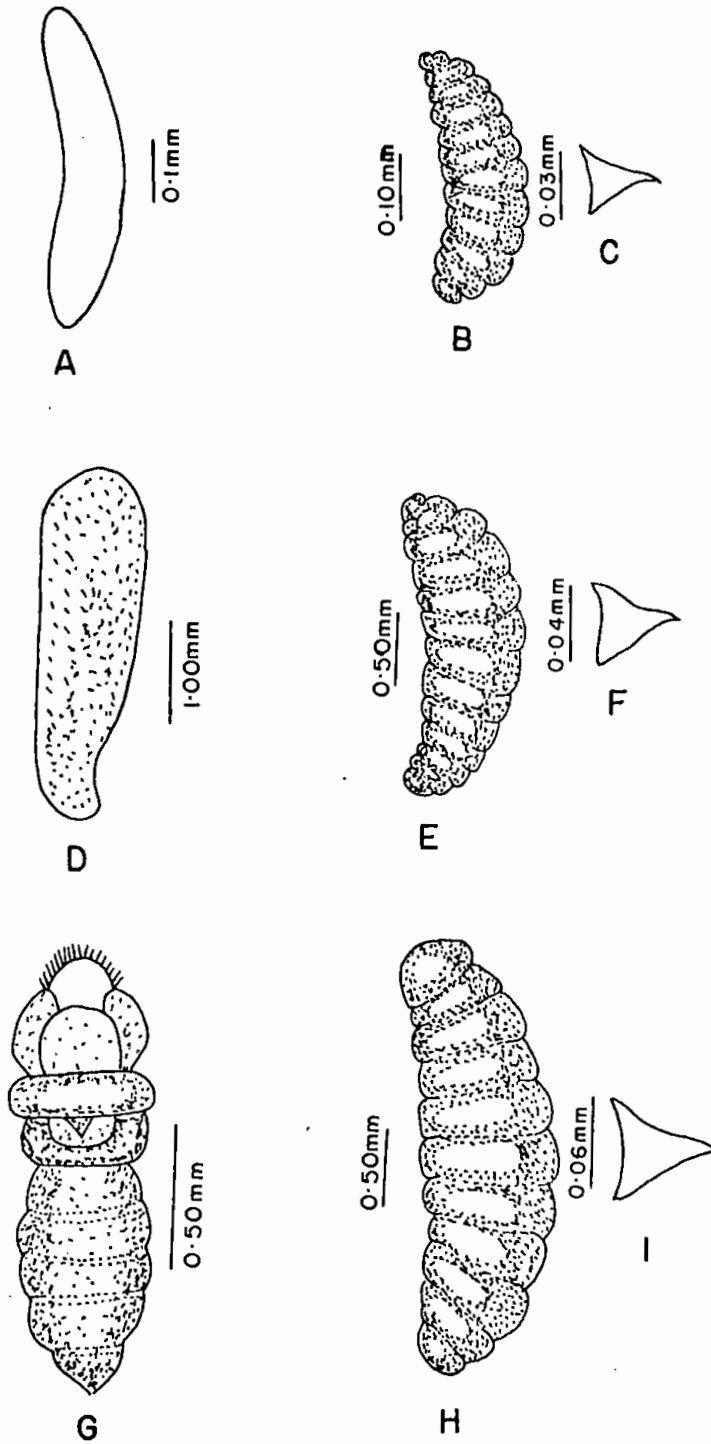


Figure 3. Development stages of *H. sylvanidis* :

- | | | |
|---------------------|---------------------------------|--|
| A. Freshly laid egg | B. 1 st instar larva | C. Mandible of 1 st instar larva |
| D. Cocoon | E. 2 nd instar larva | F. Mandible of 2 nd instar |
| G. Pupa | H. 3 rd instar larva | I. Mandible of 3 rd instar larva. |

Second instar

The second instar larva (Figures 2, 3) is hymenoteriform and the body consists of three thoracic and ten abdominal segments. It is more active and the cuticle shows a higher degree of sclerotization. The larva is yellowish-grey in colour at the mid-region of the body while the two marginal areas appear white probably due to the presence of fat bodies and muscle-fibres which give the larva a freshly appearance. The second instar larva is elongated, voracious and broader at the middle. The larva feed externally and devoured the body fluid of host.

Mandibles of the second instar larva more sclerotized than the preceding instar, each being provided with a pointed tip with a strong base. The mandible of second instar larva of *R. zae* has an average length of 0.057 mm (range: 0.048-0.064). The mandible of second instar larva of *H. sylvanidis* has an average length of 0.04 mm (range: 0.03-0.05). Similar appearances have been observed in the second instar larva of *H. sylvanidis*.

Third instar

The mature larva of 4-5 days is yellowish in colour, with numerous oval or circular discs on the entire body, and measures 2.53-2.73mm (2.66 ± 0.18) in length and 0.34 ± 0.16 mm in head capsule width. The mature larva now changes position, comes backward up to segment 7-8, lies head to head with its host, makes a circular opening and sucks vigorously the fluid content of the host larva by squeezing its body with a rapid motion. The larva becomes fullfed at the end of 4 or 5 days, thick at the middle, fat and glistening. It has a relatively small head, posterior extremity is now pointed. Mandibles (Figures 2, 3) are comparatively larger than preceding instars. The mean length of the mandibles of *R. zae* is 0.082 (range: 0.075-0.089). The mean length of the mandibles of *H. sylvanidis* 0.06 (range: 0.05-0.06).

Table 4. Length and breadth of different stages of *R. zea* (mm).

Stage	Length			Breadth		
	N	Range	Mean±SE	N	Range	Mean±SE
Egg	10	0.50-0.53	0.51±0.03	10	0.12-0.14	0.13±0.02
Larva						
1 st instar	10	0.41-0.43	0.42±0.02	10	0.11-0.13	0.12±0.02
2 nd instar	10	1.65-1.68	1.66±0.03	10	0.60-0.65	0.62±0.06
3 rd instar	10	2.53-2.73	2.66±0.18	10	0.94-0.97	0.95±0.03
Pre-pupa	10	3.48-3.52	3.50±0.04	10	1.00-1.07	1.04±0.07
Pupa						
Male	10	1.58-1.62	1.59±0.04	10	0.63-0.67	0.64±0.03
Female	10	1.90-1.97	1.93±0.94	10	0.80-0.85	0.82±0.04
Adults						
Male	8	2.60-2.67	2.64±0.06	8	0.50-0.57	0.54±0.03
Female	8	3.10-3.14	3.12±0.03	8	0.60-0.49	0.64±0.01

Table 5. Length and breadth of different stages of *H. sylvanidis* (mm)

Stage	Length			Breadth		
	N	Range	Mean±SE	N	Range	Mean±SE
Egg	10	0.46-0.50	0.47±0.04	10	0.10-0.13	0.11±0.03
Larva						
1 st instar	10	0.38-0.42	0.40±0.04	10	0.10-0.12	0.10±0.02
2 nd instar	10	1.48-1.52	1.50±0.03	10	0.58-0.62	0.60±0.03
3 rd instar	10	2.48-2.52	2.50±0.03	10	0.78-0.82	0.80±0.04
Pre-pupa	10	3.28-3.32	3.30±0.03	10	0.95-0.99	0.97±0.04
Pupa						
Male	10	1.48-1.52	1.50±0.03	10	0.48-0.52	0.50±0.04
Female	10	1.78-1.82	1.80±0.04	10	0.68-0.72	0.70±0.04
Adults						
Male	8	1.98-2.10	2.03±0.01	8	0.42-0.45	0.44±0.03
Female	8	2.50-2.60	2.54±0.01	8	0.50-0.55	0.52±0.06

Pre-pupa

The full-fed *R. zea* larva is now very restless, it wriggles about and moves to and fro in order to find a safe place for cocoon formation. After a short period, it forms a thin silver white cocoon inside the bore of the infested wheat kernal. The threads of the cocoon is delicate and very tough, it measures about 3.50 mm in length and is slightly broader interiorly near this end a circular opening is made by cutting through mandibles of the emerging parasitoid. The pre-pupa is elongated, the head region beings much narrower than the body. It is visible under the thin layer of cocoon with distinct reddish mandibles. The pre-pupal period lasts for 3-4 days.

After feeding actively *H. sylvanidis* larvae became detached from the host-body and curved and nearly inactive. The colour changed to white and the thoracic segments became constricted. Dorsally a small seta developed on each segment. The head bore one –segmented horn-like antenna. The last segment possessed four setae.

Pupa

R. zea pupation takes place within the cocoon. The freshly formed pupa is whitish, which gradually turns black before adult emergence. Then it resembles the adult in shape and size. The male pupa measures 1.59 ± 0.04 mm in length and about 0.53 mm in head capsule width. While a female pupa measures 1.93 ± 0.94 mm in length and about 0.60 mm in head capsule width. Pupal period was 9-10 days.

H. sylvanidis pupa appeared whitish at the early stage. In about 8h it changed to light brown. The eyes became red after 42h. The head and thorax appeared black in about 78h.

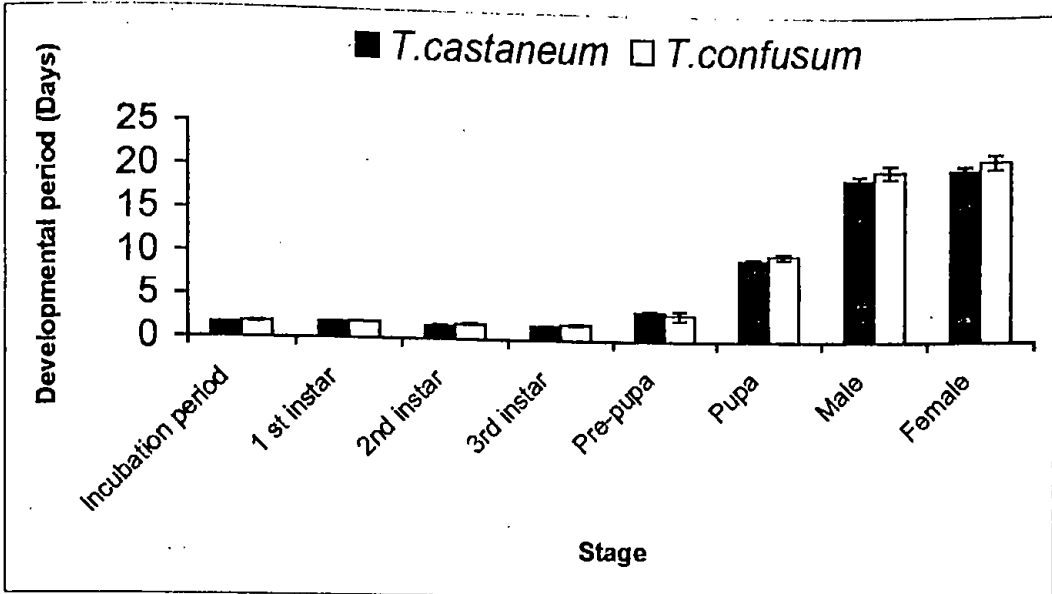


Figure 4. Mean \pm SE developmental period (days) from oviposition to adult emergence of *R. zeae* on *T. castaneum* and *T. confusum*.

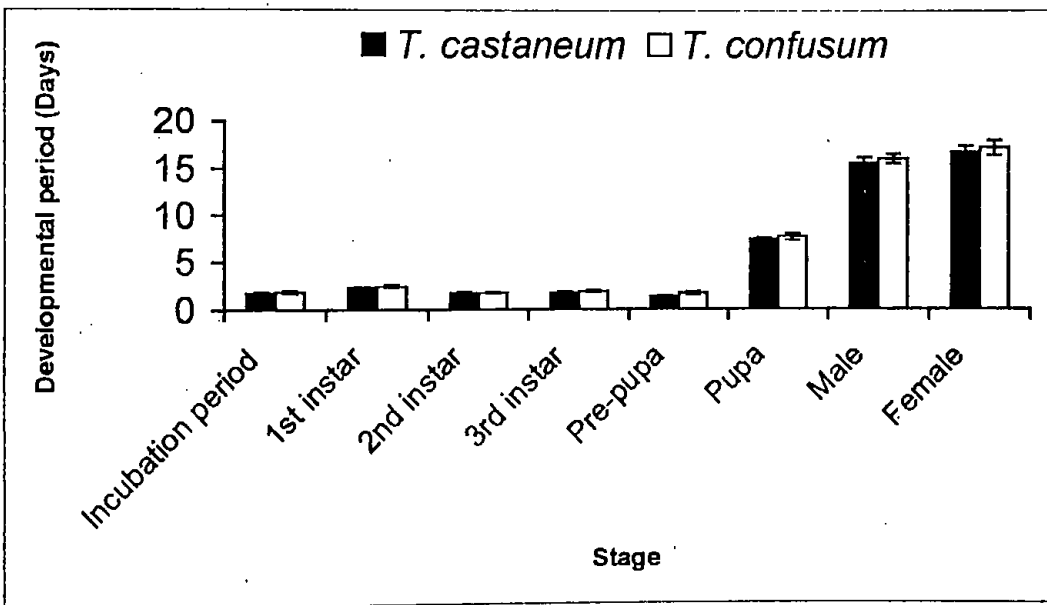


Figure 5. Mean \pm SE developmental period (days) from oviposition to adult emergence of *H. sylvanidis* on *T. castaneum* and *T. confusum*

Adults

The adult female *R. zae* is black, abdomen is brownish with pale yellow patches and measures about 3.13mm in length. The wing expanse is 1.5mm. The adult male is shiny black with numerous reddish spines and smaller than the female. It measures about 2.66 mm in length, the wing expanse is 1.33 mm. The antenna is 13 segmented and the adult parasitoid is an active flyer.

The adult *H. sylvanidis* female is yellowish. Abdomen is brownish with yellow patches, dorsal part of head and thorax with black strips, yellowish ventrally. Abdomen is rounded, swollen above with short pointed ovipositor. The adult male is with yellowish head and thorax. Abdomen is slender, cylindrical and metallic black.

R. zae and *H. sylvanidis* are arrhenotokous. Unfertilized eggs develop parthenogenetically to male progeny. The requirements and behaviour of the virgin parasitoids were similar to those of her fertilized sisters.

Xylocoris flavipes

The biology of *X. flavipes* and their role in regulating populations of storage pests has been largely neglected. It is important that this role be determined, first, because such information is essential to understanding how storage ecosystems function; and second, because it must be known before the potential of these predators as biocontrol agents can be evaluated.

Egg

The newly deposited eggs are translucent milky. As development proceeds, they become pale ochreous with a faint reddish cast, and the orange-red eyes and abdominal scent glands of the nymph become visible through the chorion. The collar and outer ring of the opercular are opaque white. The egg of *X. flavipes* is elongated-oval and

blackish with yellow spots. The length of the egg is 0.78 ± 0.02 mm, or diameter at broad of point 0.32 ± 0.02 mm ($n=15$). The posterior end is broader than anterior which bears 3 evaginations. The eggs, which are scattered loosely throughout the habitat, are ellipsoidal, and the anterior end is capped by a nearly circular operculum surrounded by an expanded rim of chorion. The chorion is marked by a faint reticulate pattern of polygons. The operculum bears a prominent tubercle at its center, which, in scanning electron micrographs, appears to consist of a mass of nodules that have coalesced after deposition on the chorion. Newly deposited eggs are nearly colorless except for the rim of the chorion and the tubercle, which are white. As the eggs develop, they acquire a pale brownish yellow hue, and red eyespots appear about 2 days after oviposition (30°C). Shortly before hatching, the eggs become deeply indented, and the red markings of the nymph are clearly visible. At 30°C , the eggs hatch in 4 to 5 days. The nymph emerges by forcing open the operculum, which usually remains attached to the shell by the embryonic cuticle.

Nymph

The nymphs are shining and sparsely setosa. The head is triangular, about as broad (across the eyes) as long. The thoracic nota are all transverse. The tarsi are 2-segmented with the 1st segment much shorter than the 2nd. The 3rd, 8th, and 9th abdominal terga each bear two long setae, one on either side. Those of the 3rd segment are located near the middle; the others are situated near the lateral margins. There are four pairs of dorsal abdominal scent gland openings as follows: a pair between segment 3 and 4, 4 and 5 and 5 and 6, with the openings of each pair joined by a groove, and a separated pair between segments 6 and 7 with the openings slightly mesad of the others.

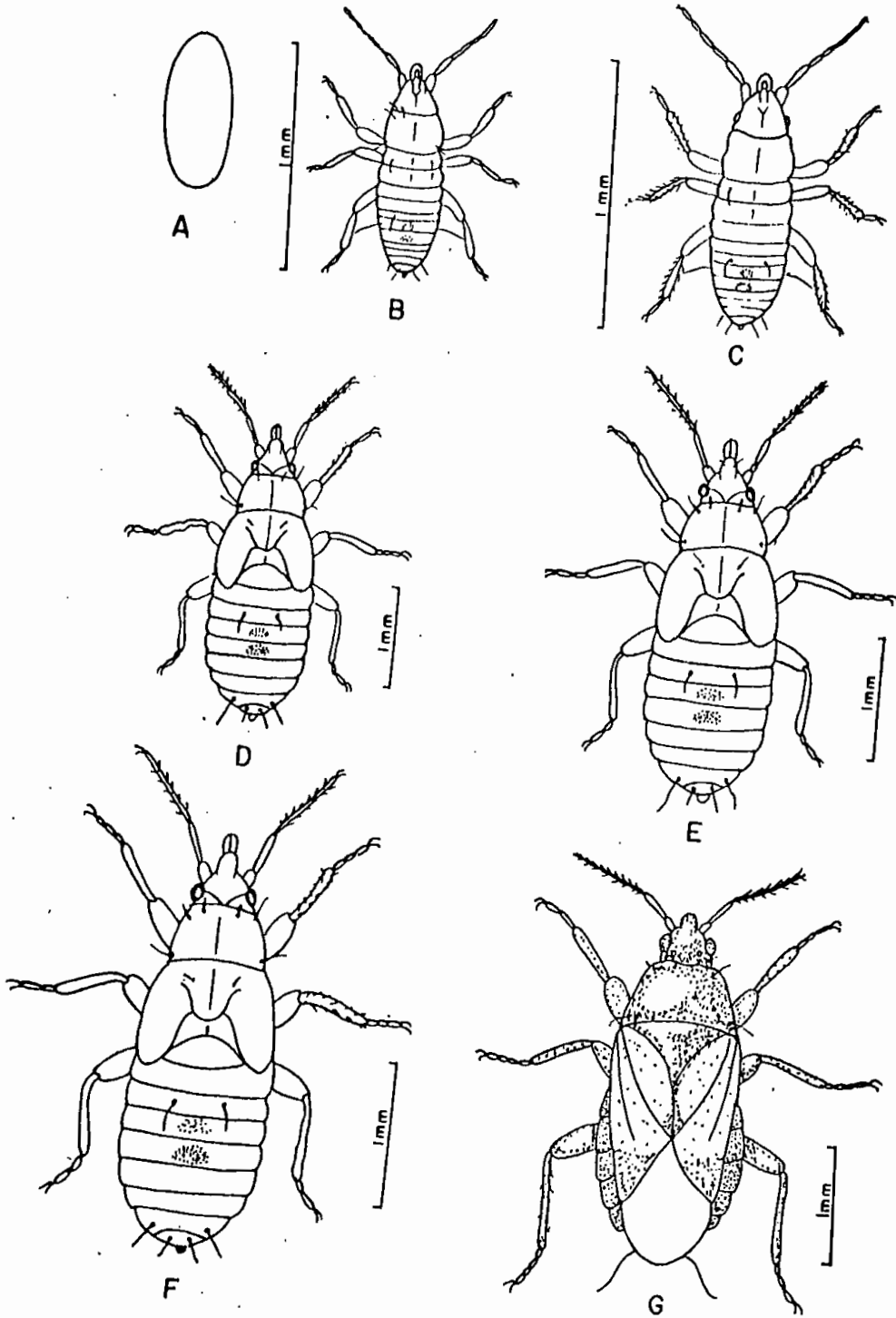


Figure 6. Development stages of *X. flavipes* :

- | | | |
|---------------------------------|---------------------------------|---------------------------------|
| A. Freshly laid egg | B. 1 st instar nymph | C. 2 nd instar nymph |
| D. 3 rd instar nymph | E. 4 th instar nymph | F. 5 th instar nymph |
| G. Adult. | | |

First instar

First-instar nymphs average 0.98 ± 0.07 mm long. The body is pale brownish-yellow slightly suffused with orange-red, especially on the head and thorax. The head and thorax are tinged with black, and the 4th and 5th abdominal terga are each marked with a median orange-red spot. The eyes are deep brick red. The legs, antennae, and rostrum are nearly colourless except for a faint black tinge on the antennae. Wing pads are lacking, and there are two long setae on the 4th abdominal tergum, one near each lateral margin. These setae are lacking in later instars.

Second instar

Essentially same color as first instar but with a heavier orange-red suffusion and occasionally with some black tinging of abdomen. Wing pads barely visible. Length 1.38 ± 0.05 , breadth 0.26 ± 0.01

Third instar

The length and breadth of the third instar nymph were 1.71 ± 0.09 and 0.31 ± 0.01 . Head, thorax, and abdomen ochreous, heavily suffused with orange red, especially head and thorax. Developing ocelli appear as small orange red spots. Abdominal scent glands orange-red to fuscous. Legs, antenna, and rostrum pale ochreous, nearly colorless. First three antennal segments and femora tinged with black. Anterior portion of head and thorax, infuscata. Lateral portions of abdominal segments infuscate. Meso- and metathoracic wing pads evident; tips of mesothoracic wing pads not extending beyond mid posterior margin of metanotum.

Fourth instar

Head, thorax, and abdomen ochreous, suffused with orange-red. Anterior portion of head pronotum, wing pads, and all or part of abdomen infuscate. Infuscation of abdomen usually lighter or absent on first two segments, giving the appearance of a

light band across the body at this point. Infuscation often limited to lateral portions of last two segments, giving the appearance of a high patch in this area. Egg deep brick red. Abdominal scent glands fuscous. Legs, antennae and rostrum ochreous. Wing pads extending to abdominal segment. Length 2.13 ± 0.14 , breadth 0.38 ± 0.01 .

Table 6. Length and breadth of different stages of *X. flavipes* (mm)

Stage	Length			Breadth		
	N	Range	Mean \pm SE	N	Range	Mean \pm SE
Egg	15	0.77-0.79	0.78 \pm 0.02	15	0.31-0.33	0.32 \pm 0.02
Larva						
1 st instar	13	0.97-0.99	0.98 \pm 0.07	13	0.20-0.23	0.22 \pm 0.00
2 nd instar	13	1.35-1.39	1.38 \pm 0.05	13	0.22-0.27	0.26 \pm 0.01
3 rd instar	18	1.70-1.72	1.71 \pm 0.09	18	0.30-0.31	0.31 \pm 0.01
4 th instar	15	2.10-2.14	2.13 \pm 0.14	15	0.36-0.39	0.38 \pm 0.01
5 th instar	17	2.60-2.66	2.65 \pm 0.17	17	0.42-0.45	0.44 \pm 0.01
Adults						
Male	8	2.82-2.84	2.83 \pm 0.13	8	0.42-0.45	0.45 \pm 0.01
Female	8	2.97-2.99	2.98 \pm 0.10	8	0.46-0.49	0.48 \pm 0.01

Fifth instar

Fifth instar nymphs are dark brownish yellow to brown with pale brownish yellow legs, antennae and rostrum. The head and thorax, except for the scutellum, are tinged with black especially on the wing pads. The abdomen may also be tinged with black and marked by a row of diffuse black spots on either side of the dorsum, but such black colouration is often faint or absent. In dark nymphs the median abdominal spots appear reddish-brown. In light nymphs they are orange red as in earlier instars. Length 2.65 ± 0.17 , Breadth 0.44 ± 0.01 .

Development of the wing pads provides the most reliable method of separating instars 2-5. Wing pads first appear in the 2nd instar as barely perceptible protrusions on the latero-posterior margins of the meso- and meta thoracic nota. Mesothoracic wing pads are well developed in the 3rd instar but extend only slightly beyond the mid-posterior margin of the mesonotum. In the 4th instar, they extend well beyond this margin and in the 5th instar they reach the tips of the metathoracic pads.

Adults

The adults are shining brownish black with sparsely distributed pale setae. The rostrum, the antennae, legs and hemelytra are brownish-yellow. The cuneus, the distal portion of the corium, and the inner margin of the clavus are tinged with black. The membrane is hyaline and without veins. The compound eyes are dark brown and the ocelli are red. The pronotum is somewhat convex with immarginate sides and very slightly rounded anterior angles. The scutellum is raised anteriorly. The antennae are 4-segmented with the 3rd and 4th segments much thinner than the other two and clothed with long erect setae. The rostrum and tarsi are 3-segmented.

The sexes can be distinguished by the shape of the abdomen, which is bilaterally symmetrical in females but in males is notched on the left side of segments 8 and 9. The aedeagus arises within the 9th segment and is directed to the left.

Brachypterous and macropterous forms occur in both sexes. The hemelytra of brachypterous adults do not reach the posterior margin of the 4th abdominal segment; those of the macropterous form always extend well beyond this segment and may reach the tip of the abdomen if feeding has not distended the abdomen. The brachypterous form is by far the more common of the two and in a sample of 105 adults 46% were brachypterous males, 42% brachypterous females, 3% macropterous males and 9% macropterous females.

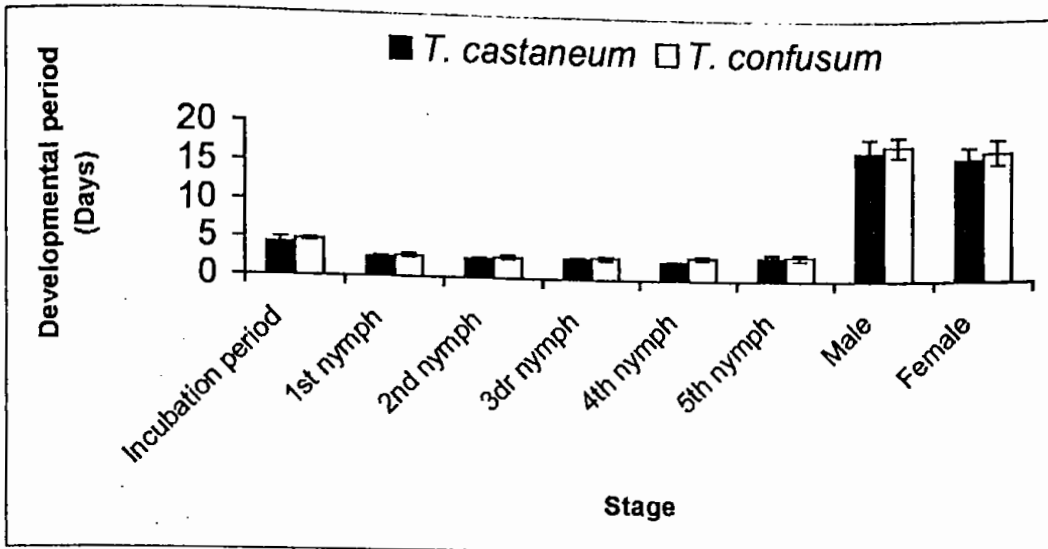


Figure 7. Mean \pm SE developmental period (days) from oviposition to adult emergence of *X. flavipes* on *T. castaneum* and *T. confusum*.

As in most anthocorids, copulation in *X. flavipes* is extragenital traumatic (Carayon 1972) and seminal stimulus is necessary for normal egg development (Carayon 1970). The aedeagus of the male, which is sclerotized and sharply pointed, pierces the inter-segmental membrane between the 2nd and 3rd abdominal tergites of the female. Sperm are deposited directly into the haemocoel and migrate to the ovaries. The points of penetration are marked by plugs of tanned cuticle (copulation scars) that can be observed readily in specimens mounted that can be observed readily in specimens mounted and cleared in polyvinyl alcohol lactic acid-phenol.

Developmental period

The mean developmental period from oviposition to adult emergence at $30 \pm 1^\circ\text{C}$ and $70 \pm 0.05\%$ relative humidity did vary between males and females in *R. zae*, *H. sylvanidis* and *X. flavipes*. The mean developmental period of male of *R. zae*, *H. sylvanidis* and *X. flavipes* on *T. castaneum* were 19.06 ± 0.59 days (range: 17.90-20.04), 15.50 ± 0.62 days (14.50-17.00) and 16.99 ± 1.86 days (range: 15-21), respectively. The mean developmental period of male of *R. zae*, *H. sylvanidis* and *X. flavipes* on *T.*

confusum were 20.10 ± 0.77 days (range: 18.50-21.80), 16.00 ± 0.47 days (15.00-17.00) and 17.80 ± 1.30 days (range: 16.00-21.00), respectively. The mean developmental period of female of *R. zea*, *H. sylvanidis* and *X. flavipes* on *T. castaneum* were 20.14 ± 0.58 days (range: 18.90-21.40), 16.66 ± 0.72 days (range: 15.00-18.00), 16.00 ± 1.63 days (range: 14-20) respectively. The mean developmental period of female of *R. zea*, *H. sylvanidis* and *X. flavipes* on *T. confusum* were 21.20 ± 0.92 days (range: 19.00-22.80), 17.15 ± 0.80 days (range: 15.50-18.70), 17.00 ± 1.63 days (range: 15.00-20.00) respectively.

Discussion

The published materials on *R. zea* and *H. sylvanidis* did not adequately provide detailed information regarding biology of both the bethylids. The present investigation deals with more details of the biology on the host *Tribolium*.

The egg of *R. zea* and *H. sylvanidis* found to be typical hymenopterian form. It is elongated, slightly broader and blunter at the head end. Ahmed and Islam (1988) described the egg of *R. zea* is elongated and broader at the posterior portion. It is whitish and gradually changing into pale yellowish at the central portion before hatching. The larva of both the bethylids has three instars. The descriptions of larval instars, pre-pupa and pupa of Ahmed and Islam (1988) agree well with the present findings.

The immature stages of *R. zea* and *H. sylvanidis* may be easily distinguished by their shape and size. Individual larval instars of *H. sylvanidis* are slightly smaller than those of *R. zea*. The most striking distinguishable characters are the mandible which vary in shape and size between these two species. Besides, the relative length, width and position of different organs differ between the two species. So, not only biological characteristics, morphological features may be used for their identification.

Arbogast *et al.* (1971) and Awadallah and Tawfik (1972) described the developmental stage of *X. flavipes* reared on different hosts. Arbogast (1979) evaluated the

morphology of immature stages of *X. flavipes* reared of stored product pests but this information is not enough for finally biological parameters of *X. flavipes*. Awadallah and Tawfik (1972) reported that there is usually five nymphal instar of *X. flavipes*, though the number ranges from 2 to 6. Arbogast (1979) found *X. flavipes* have five nymphal instar. The present investigation reported the same observation of Arbogast (1979).

Arbogast (1979) described the egg of *X. flavipes* as ellipsoid, curved near anterior end. It is white in colour measuring 0.77 ± 0.02 mm in length and 0.31 ± 0.02 mm in breadth. In the present investigation it has been found that the egg is elongated-oval and blackish. The chorion is marked by a faint reticulate patten of polygons. Newly deposited eggs are nearly colourless except for the rim of the chorion are the tubercle. Arbogast *et al.* (1985) reported that the egg of *X. sordidus*, a predaceous bug that occurs in storage ecosystem is ellipsoid, curved near anterior end, with a prominent collar surrounding the opercular which are related in the present observation.

The first instar nymph with pale brownish-yellow slighty with orange-red, especially on the head and thorax. The eyes are deep brick red. Wings are lacking. Arbogast (1979) found the first instar of *X. flavipes* having pale brownish-yellow lightly suffused with orange-red, especially on the head and thorax. The head and thorax are tinged with black, and the 4th and 5th abdominal terga are each marked with a median orange-red spot. Arbogast *et al.* (1985) found that the first instar nymph of *X. sordidus* has head, thorax, and abdomen pale ochreous suffused with orange-red. Eyes and abdominal scent glands are orange-red. Legs, antennae, and rostrum nearly colorless. Head, thorax, and first three antennal segments tinged with black.

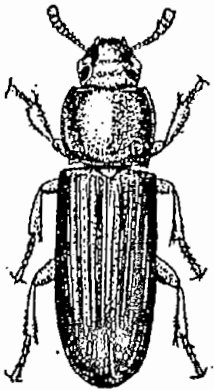
The second instar nymph of *X. flavipes* also pale brownish-yellow with orange-red, on the head and thorax. Wings pads barely visible. Similar observations were observed by Arbogast *et al.* (1971) and Arbogast *et al.* (1985) in *X. sordidus*.

Third instar nymph are comparatively larger than the preceding instars. Its head, thorax, and abdomen ochreous. Abdominal scent glands orange-red to fuscous. The first three antennal segments and femora tinged with black. Lateral portions of abdominal segments infusate. The third-instar larva possess small orange red spot ocelli and legs, antenna, and rostrum pale ochreous, nearly colorless in *X. sordidus* (Arbogast *et al.* 1985). Arbogast *et al.* (1971) found meso and metathoracic wing pads evident.

The fourth instar nymph of *X. flavipes* are more or less similar with that of third instar. Similar finding were observed by Arbogast *et al.* (1985) and Arbogast *et al.* (1971).

The fifth instar nymph larger than preceding instar. Head, thorax and abdomen ochreous, suffused with orange-red. Anterior portion of head (frons, tylus, juga), pronotum (except, occasionally for media portion), wing pads, and all or part of abdomen infusate, similar finding in *X. sordidus*.

Arbogast (1975) reported that the development of *X. flavipes* was completed in 14-24 days at 30°C when *Plodia interpunctata* (Hubner) was host. The mean developmental time at 30 and 35°C different only slightly from that (17.2 days) found by Awadallah and Tawfik (1972) when they reared *X. flavipes* on *Tribolium* larvae at a room temperature that ranged from 28 to 34°C temperature.



Chapter 7

Effect of different foods on adult longevity

Effect of different foods on adult longevity

Adult longevity is important in enabling hymenopteran parasitoids to find more hosts as well as to disperse in a higher degree in the environment. Thus, it plays a significant role in the biological activities of parasitoids. Stary (1970) stated that a number of factors such as temperature, relative humidity, photoperiod, season, food, sex, host density, mating, etc. have a significant effect on adult longevity.

So an attempt was made to study the adult longevity of *R. zea*, *H. sylvanidis* and *X. flavipes* in relation to the effect of different foods.

Materials and methods

Pupae of the parasitoids and last nymphal stages of the predator were collected from the culture and kept in a small separate beakers (250 ml) until adult emergence. After emergence a male and female were paired and placed in a glass vial (15 cm) for subsequent mating. In this way several mated males and females were obtained. The adult longevity was determined under five experimental conditions e.g. honey, glucose, sucrose, distilled water, host and control (with out food).

Control was also maintained without providing any commodity. For each type of food, four replications were maintained. The adults were kept with different foods in separate glass tubes and placed in incubator at 30°C. The food solutions were supplied to each of the experimental tubes in wads of cotton twice daily with the help of a glass dropper. The wads of cotton were removed after two days interval to avoid any fungal infection.

In case of host supplied, the mated female was provided with 20 hosts in different petri dishes (8.5 cm diam.). This procedure was repeated until all the female parasitoids and predator died.

The mortality of the parasitoids was recorded at 6-h intervals. The data were subjected to analyses of variance (ANOVA). The experiments were carried out at $30 \pm 1^\circ \text{C}$ and $70 \pm 0.5\%$ relative humidity.

Results

The effects of different types of food on the adult longevity of both male and female of *R. zaeae*, *H. sylvanidis* and *X. flavipes* are well documented in Figures 8-10. The data indicated that the two parasitoids and one predator in both sexes had comparatively shorter longevity in starved condition. Among all food items tested, the parasitoids and predator lived longest in both sexes when the host was supplied. In all the cases the mean longevity of males was less than that of females. Significant difference in longevity between the two sexes were noticed ($P < 0.001$).

R. zaeae

Maximum longevity was observed when host (*T. castaneum*) was supplied as food, the longevity of male and female were 10.85 (range: 6-14) and 19.85 (range: 18-22) days and host (*T. confusum*) was supplied as food the longevity of male and female were 9.85 (range: 4-10) and 19.20 (range: 14-20) days, respectively. Honey ranked the second highest food was provided in both the sexes 10.6 (range: 6-15) and 19.7 (range: 8-30) days, respectively. Glucose ranked the third highest food that exhibited mean longevity of 8.75 (range: 6-12) and 12.85 (range: 9-20) days, respectively for male and female. Sucrose and distilled water when provided as food mean longevity of male and female were more or less same, 6.25 (range: 5-10) and 8.4 (range: 6-10) days, and 6.05 (range: 5-9) and 10.33 (range: 6-14) days, respectively. Minimum longevity was observed when both the sexes were starved 2.35 (range: 2-3) and 5.1 (range: 4-6) days, respectively.

Analysis of variance shows that the adult longevity of *R. zaeae* was significantly ($P < 0.01$) affected by different foods, sex and dose. Among the interaction between sex \times dose was highly significant ($P < 0.01$) (Appendix Table 7).

H. sylvanidis

The adult longevity of *H. sylvanidis* on various types of foods showed that males and females had comparatively longer longevity when hosts (*T. castaneum* and *T. confusum*) were supplied continuously, 7.9 (range: 5-12) and 7.00 (range: 5-12), and 18.10 (range: 16-20) and 17.2 (range: 17-21) days, respectively. Honey was the next rank for longevity of the parasitoid in both the sexes, 7.24 (range: 5-14) and 16.7 (range: 8-28) days, respectively. Glucose and sucrose ranked the second and third foods for longer survival of *H. sylvanidis*, and were 7.20 (range: 5-13) and 14.95 (range: 7-22), and 6.15 (range: 4-10) and 11.25 (range: 7-16) days, respectively. Both males and females survived for 1.85 (range: 1-3) and 2.95 (range: 2-5) days, and 5.75 (range: 4-9) and 10.0 (range: 6-14) respectively in starved and distilled water condition.

Foods, sex and dose significantly affected the adult longevity of *H. sylvanidis* ($P < 0.01$). Interaction between sex x dose was highly significant ($P < 0.01$) (Appendix Table 8).

X. flavipes

The adult longevity of both males and females of *X. flavipes* indicated that the predator achieved maximum longevity when host (*T. castaneum* and *T. confusum*) were supplied, 7.05 (range: 4-14) and 6.25 (range: 4-12), and 37.2 (range: 30-45) and 36.15 (range: 30-43) days, respectively. The next highest food was honey, 8.66 (range: 4-15) and 24.1 (range: 20-26) days, respectively. The third highest food was glucose for longevity of *X. flavipes*, 7.45 (Range: 5-15) and 20.13 (range: 17-25) days, respectively. When sucrose was provided as food the mean longevity of the adult males and females were 6.9 (range: 4-14) and 18.95 (range: 15-21) days, respectively. When distilled water supply the adult longevity was male 6.35 (range: 2-12) and 17.5 (range: 15-21) days, respectively. Minimum longevity was recorded in starved condition.

Foods significantly ($P < 0.01$) affected the longevity of *X. flavipes*. Sex, replications and dose were highly significant ($P < 0.01$). Sex x dose interaction was also highly significant ($P < 0.01$) (Appendix Table 9).

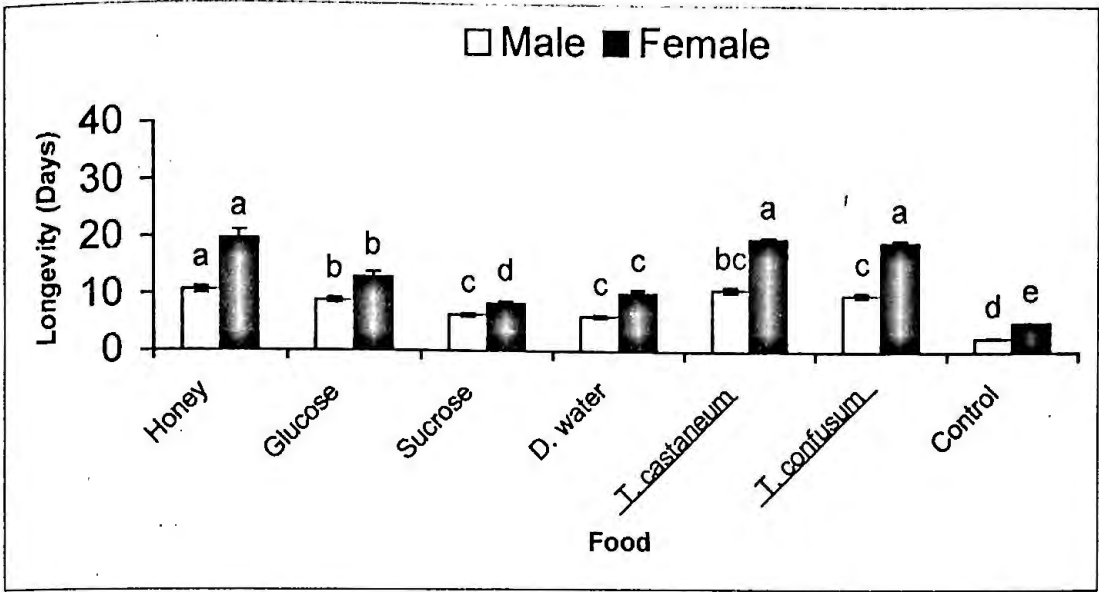


Figure 8. Mean \pm SE adult longevity (in days) of *R. zae* fed on different types of food.

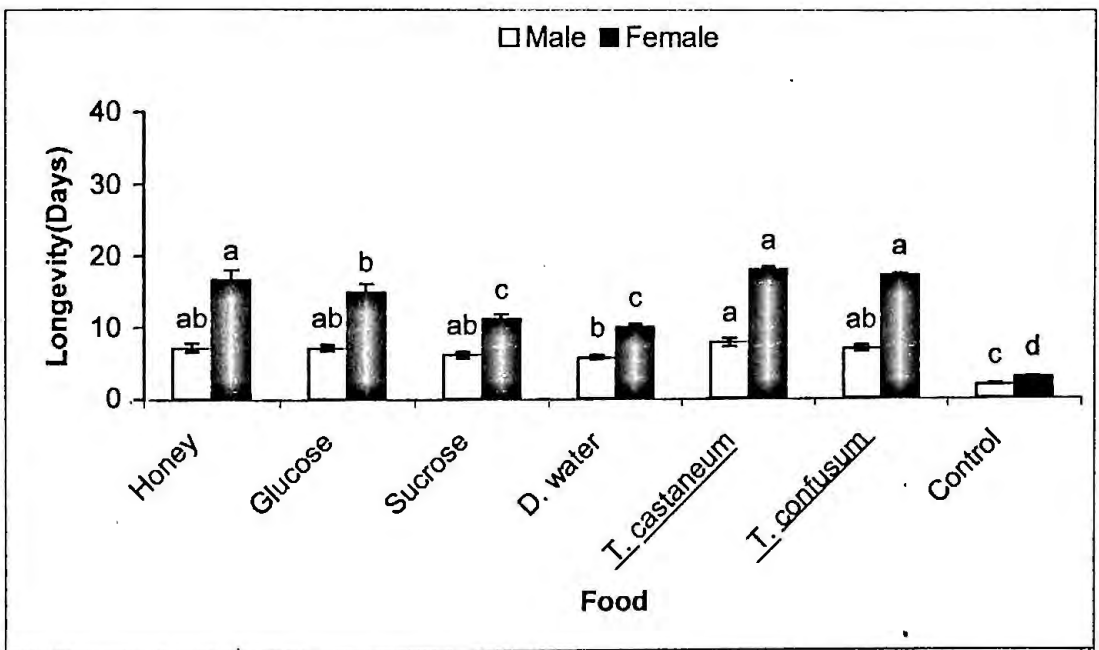


Figure 9. Mean \pm SE adult longevity (in days) *H. sylvanidis* fed on different types of food.

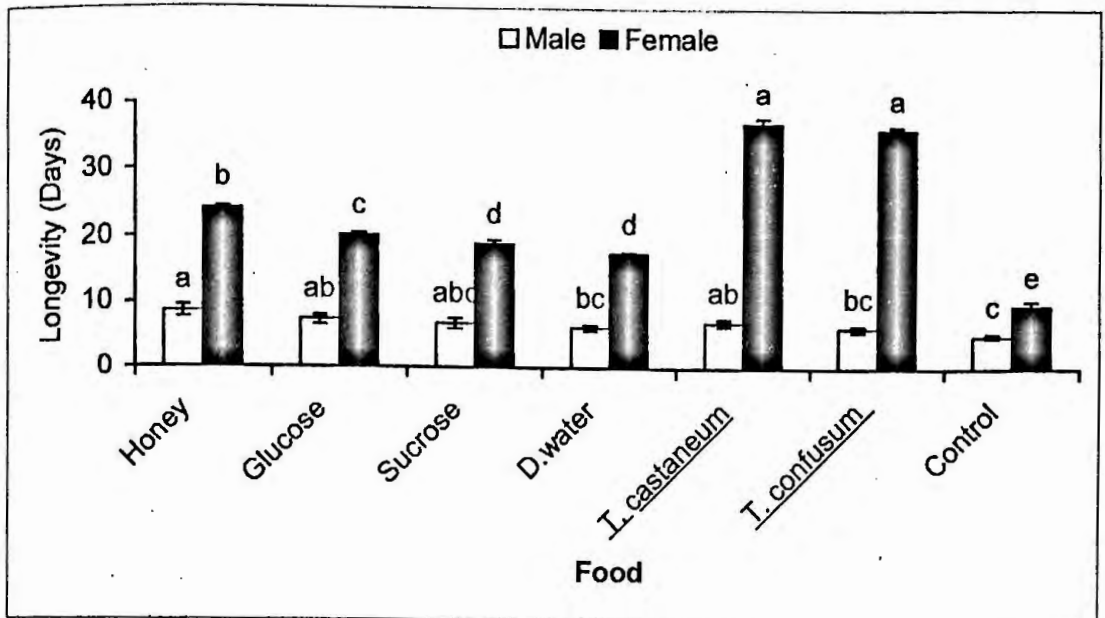


Figure 10. Mean \pm SE adult longevity (in days) *X. flavipes* fed on different types of food.

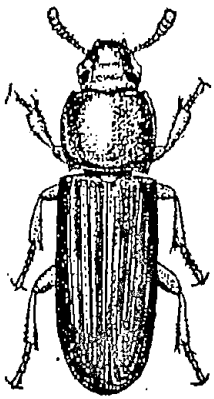
Discussion

Diet at the adult stage has a great impact on the longevity of the adult insects and regulates the biology to some extent. Adult lifespan in the laboratory varied widely according to the rearing temperature, and to the diversity of food and hosts that were provided. According to Andrewartha and Birch (1954) both the longevity and reproductive potentiality are strongly influenced by the components of the environment other than temperature, notably moisture and food. Published information on the effect of adult diet on the longevity of *R. zea*, *H. sylvanidis* and *X. flavipes* are scanty. In all cases the adult longevity were longest when host was supplied. Similar results were reported by other authors (Menon *et al.* 1961, Awadallah and Tawfik 1972, Arbogast *et al.* 1977, Arbogast 1975, Ahmed and Islam 1988, Flinn and Hagstrum 1995).

Awalladah and Tawfik (1972) found the mean adult lifespan (21.6 days) and oviposition period (17.5 days) of *X. flavipes*, which are closely related to the present study. Arbogast *et al.* (1977) found highest longevity of *X. flavipes* at 30°C and 74% R.H.

Use of extra-host resources such as honeydew, pollen, nectar and other plant exudates for adult longevity has been little reported in bethylids. Yadav (1985) observed *G. nephantidis* feeding on the nectar of shrub flowers. Similarly, *C. stephanoderis* appears to be attracted to the flowers of weeds near coffee plants and female longevity increase when they are provided with *Euphosbia hista* L. flowers

Honey significantly increased male and female parasitoids survival time than host fluids in all the cases in the present study. Different pteromalids fed with honey achieved maximum longevity as reported by several authors (Wylie 1963, 1964, 1976).



Chapter 8

Effect of different foods on fecundity

Effect of different foods on fecundity

Fecundity is the innate potential reproductive capacity of an individual organism, as denoted by its ability to form and separate from the body, the mated germ cells (Lapedes 1974). Availability of resources to the adult may influence the reproductive performance in several ways in many insects' species (Johansson 1964). In biological control applications, food and habitats are very important in choosing a potentially beneficial organism. The parasitic Hymenoptera, which are used as important biological control agents, require available foods for survival and reproduction. In nature, in nectars, pollens, honeydews and other plant exudates in the field are the major source of food for most adult hymenopteran parasitoids (Leius 1961a, b) but in the laboratory honey and sugar solutions are the main substitute foods (DeBach and White 1960, Lum 1977). Stary (1970) also mentioned that a number of factors like the age of the female, mating, food, mortality of progeny, size, host preference, host density, temperature, photoperiod, season may influence the fecundity. The present experiment was conducted to assess the influence of different foods on fecundity of parasitoids and predator *R. zae*, *H. sylvanidis* and *X. flavipes*.

Materials and methods

Freshly emerged mated females were collected from the laboratory culture and subsequent mating was observed. These females were released separately for oviposition in different petri dishes containing single larva. In case of *X. flavipes* mated females were individually supplied with 20 fresh and healthy larvae, in different petri dishes.

All the petri dishes were kept in the incubator at the desired temperature. The parasitized larvae of the mated females were removed from the incubator every 24 hrs for the supplied of unparasitized one. These procedures were repeated daily until all the females died. Subsequent foods: honey, glucose, sucrose, distilled water were supplied

in wads of cotton and host (*T. castaneum* and *T. confusum*) supplied individually. The parasitoids/predator were placed in the incubator and the foods were replaced everyday. The potential fecundity of the parasitoids/predator on different food sources was assessed. The larvae were observed and the total numbers of eggs deposited were counted daily. Data were obtained for 20 mated females separately and were replicated four times. The data were analysed using analysis of variances (ANOVA). All the observations were conducted at $30\pm 1^{\circ}\text{C}$ and $70\pm 0.05\%$ R.H.

Results

Results of the total production of eggs of *R. zaeae*, *H. sylvanidis* and *X. flavipes* after feeding honey, glucose, sucrose, distilled water, host and control are presented (Figures 11-13). The figures indicated that host fluids are necessary for higher egg production in all the parasitoids/predator evaluated.

R. zaeae

When *T. castaneum* was supplied as food *R. zaeae* produced maximum number of eggs and it was 104.05 ± 1.79 (range: 88-115) (Figure 11) but when provided with *T. confusum*, the egg production was slightly lower 102.55 ± 1.74 (range: 88-113). The second highest fecundity was 80.95 ± 2.04 (range: 65-97) when *R. zaeae* was provided with honey solution as food, glucose was the next highest for egg production and it was 63.35 ± 0.92 (range: 55-68). Egg productions were more or less similar in case of sucrose or distilled water. In case of sucrose it was 50.35 ± 0.63 (range: 46-55) and 49.30 ± 1.27 (range: 40-56) in case of distilled water. In starved condition the minimum fecundity was 39.05 ± 1.56 (range: 28-50).

Food played a major role for egg production, which found to be highly significant ($P < 0.001$) (Appendix Table 10). It indicates that different types of foods are unequal for egg production.

H. sylvanidis

The mean total eggs produced by female *H. sylvanidis* are presented in Figure 12. Like *R. zea*, host was the main food for production of eggs. When *T. castaneum* was supplied as food the total production of egg of *H. sylvanidis* was 78.30 ± 1.64 (range: 64-88) but it was slightly lower when *H. sylvanidis* was provided as food, *T. confusum* 76.68 ± 1.65 (range: 64-89). When honey was provided as food the second highest fecundity was 61.2 ± 1.79 (range: 48-76). The 3rd highest number of eggs 48.2 ± 1.04 (range: 40-56) when glucose was provided as food. The eggs production gradually declined when the female was fed with distilled water 40.25 ± 1.03 (range: 32-48) and sucrose 38.45 ± 0.76 (range: 32-44). In starved condition the minimum fecundity was 34.30 ± 1.35 (range: 28-48).

Analysis of variance (ANOVA) indicated that food significantly ($P < 0.001$) affected the egg production of *H. sylvanidis* (Appendix Table 11).

X. flavipes

The mean total highest number of eggs produced by the female *X. flavipes* when fed on *T. castaneum* was 98.65 ± 1.81 (range: 88-116) (Figure 13). The female produced slightly fewer eggs when fed on *T. confusum*, 97.05 ± 1.73 (range: 86-115). The second highest fecundity produced by *X. flavipes* when fed honey and it was 76.20 ± 1.18 (range: 66-84). The mated female produced 65.20 ± 0.77 (range: 60-70) eggs when glucose was provided as food. The fecundity gradually decreased 50.20 ± 1.4 (range: 40-60) and 47.40 ± 1.43 (range: 38-60) respectively when fed sucrose and distilled water. In starved condition the fecundity is 42.22 ± 1.47 (range: 28-50). (Appendix Table 12)

Significant ($P < 0.001$) differences were noticed between the egg production of *X. flavipes* when provided with different foods.

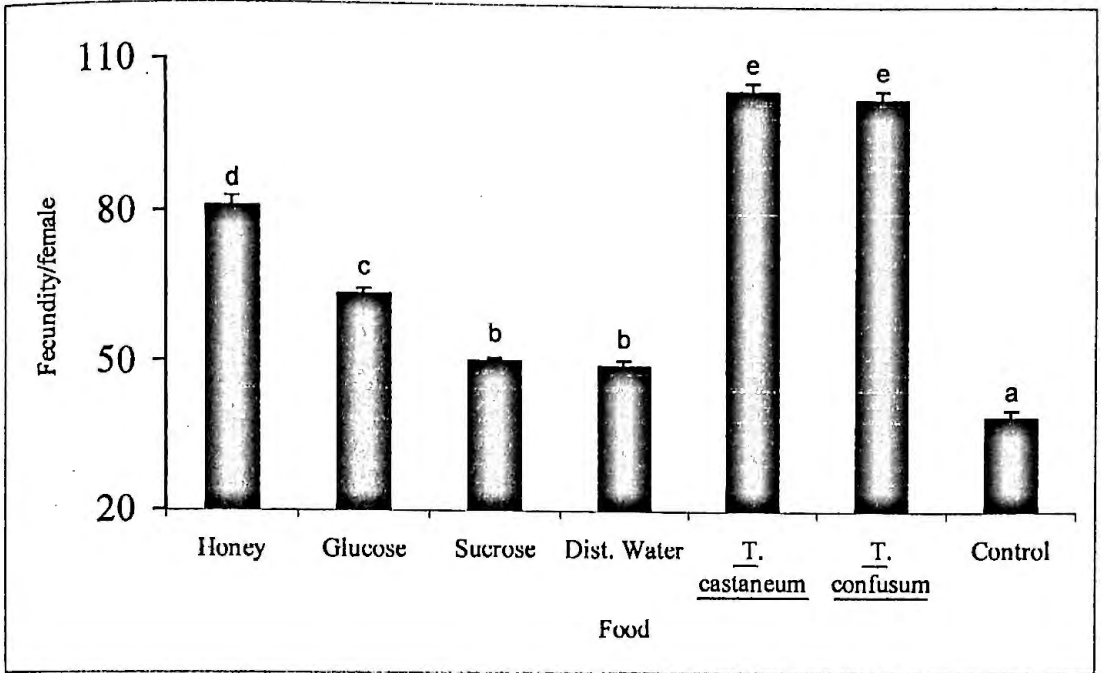


Figure 11. Mean \pm SE number of fecundity per mated female of *R. zae* fed on different foods.

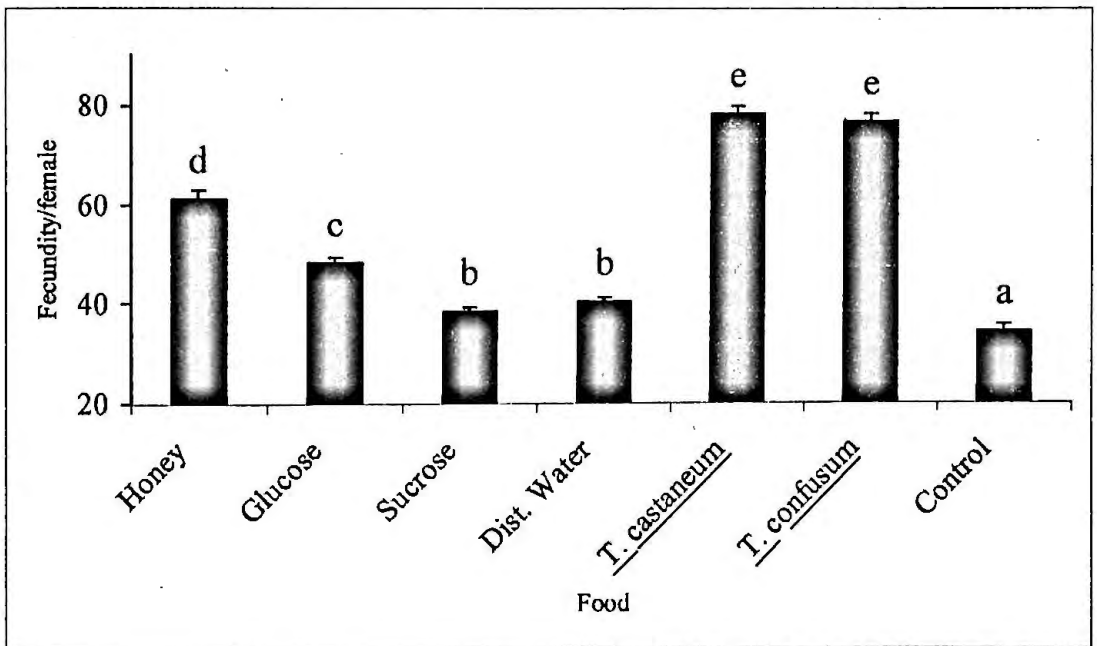


Figure 12. Mean \pm SE number of fecundity per mated female of *H. sylvanidis* fed on different foods.

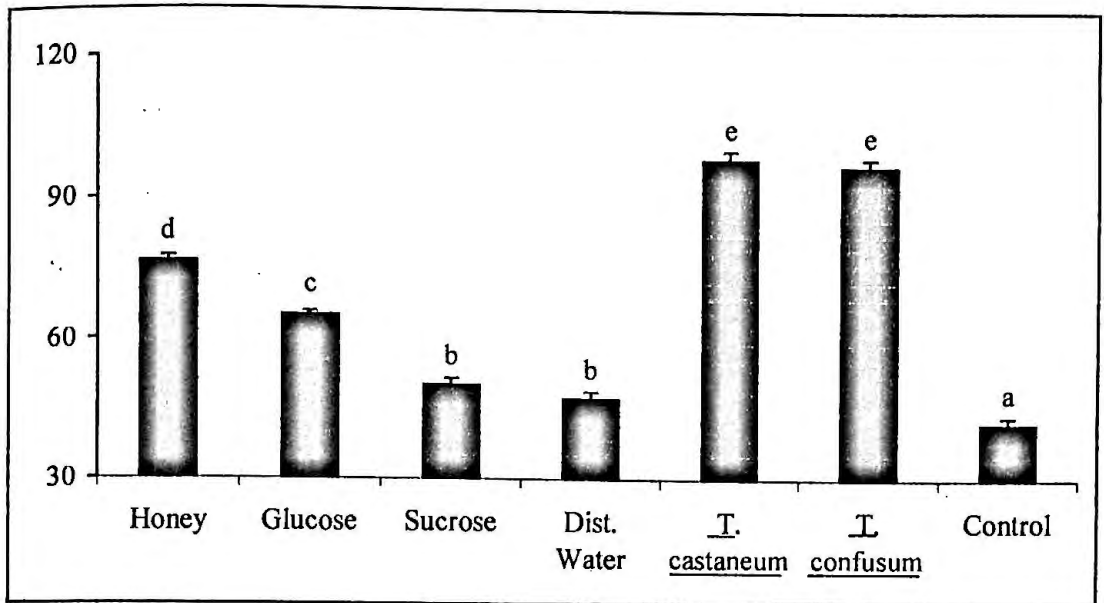


Figure 13. Mean \pm SE number of fecundity per mated female of *X. flavipes* fed on different foods.

Discussion

Differences in the nutritional requirements of various adult insects are probably related to differences in nutrient reserves accumulated during preimaginal development and utilised later by the adult (House 1961). Apparently adults of the parasitoids have low reserve and therefore, different diets have a marked effect on egg production. Most parasitic Hymenoptera continue to produce eggs throughout the adult life and they were termed as "synovigenic" by Flanders (1950). In such cases the production of eggs is depended on the nutrition of the female rather than on the metabolites retained from the immature stages. *R. zae*, *H. sylvanidis* and *X. flavipes* were synovigenic, the sequence of egg-production being cyclic and not linear. Eggs are produced throughout the adult life. Bethylids have a variable range of egg laying capacity per day depending on the availability and suitability of host's and the prevailing environmental conditions. Information in the egg production of *R. zae* and *H. sylvanidis* are not available. In the

present study, a maximum of 104.05 ± 1.79 eggs by a single mated female *R. zae* and 78.30 ± 1.64 eggs of *H. sylvanidis* are recorded which are very similar to other bethylid parasitoids. The mean lifetime fecundity of *C. hyalinipennis* is 88 eggs (Perez-Lachaud and Hardy 1999), 85 eggs in *C. tarsalis* (Blackburn 1990), 102 eggs in *C. waterstoni* (Finlayson 1950), 66 eggs in *C. stephanoderis* (Infante *et al.* 1992). The synovigenic parasitoids have very limited egg storage capacity and are able to resorb mature eggs and developing larvae when starved and deprived of hosts (Flanders 1942, Edwards 1954, King and Richard 1968, Nesar 1973).

During the present observation it was evident that diet produced notably impact on the fecundity. The mated females of *R. zae* and *H. sylvanidis* fed host fluids showed highest fecundity, host feeding has been reported in several bethylid species in addition to *C. hyalinipennis* (Bridwell 1919, Gordh *et al.* 1993). But the honey solution increases the production in different pteromalid parasitoids which was similar to that of Wylie (1962, 1963, 1964, 1976). Parthenogenesis occurs in many Hymenoptera. *R. zae*, *H. sylvanidis* and *X. flavipes* are arrhenotokous. Unfertilized eggs develop into haploid males and fertilized ones give rise to diploid females. Thus virgin females produce male offspring only. Wilson (1962) stated that in honeybee and many other hymenopteran, the fertilized eggs produce females while the unfertilized ones emerged as males (Dziezon's law).

Ali *et al.* (1981) reported that except some lepidopteran, majority of insects required full at adult stage in order to produce more eggs. The number of eggs produced varied with the type of food given (Leius 1961a). Female *Bracon hebetor*, *Aphytis lignanensis* and *Nasonia vitripennis* produced less number of eggs when starved or provided waters only (Benson 1973). Stary (1970) stated that honeydew from aphids is probably the main natural source of foods for adult parasitoids and should theoretically satisfy the nutritional requirements. Host feeding parasitoids and other synovigenic parasitoids have very limited egg storage capacity and are able to resorb mature eggs and develop larvae when starved and deprived of hosts (Flanders 1942, Edwards 1954, King and

Richard. 1968, Nesar 1973). The total number of eggs laid by a single female *X. flavipes* ranged from 1 to 8, and the mean number (\pm SD) laid was 3.0 (\pm 2.0) studied Arbogast *et al.* (1977). Arbogast (1975) reported that the lifespan and oviposition period of *X. flavipes* were longest at 20°C, but the maximum rate of oviposition occurred at 25 and 30°C. The lifespan of adult females and duration of the oviposition period decreased as temperature increased. He further reported fecundity (total eggs laid) was adversely affected by low and high temperatures, particularly high temperature. Each female of *X. flavipes* laid an average of 41.6 eggs in a lifetime reported Awadallah and Tawfik (1972), a value that differs from those observed in the present study. The present findings on the parasitoids/predator *R. zae*, *H. sylvanidis* and *X. flavipes* also confirm the above findings.



Chapter 9

Sex ratio

Sex ratio

Sex ratio partly regulates the population of species (Howe 1953, Morris and Miller 1954). Fisher (1930) predicted that there should be equal investment in male and female offspring in randomly mating populations. Generally in the offspring sex may be determined at or before conception as in male or female heterogametes. In several organisms, it is determined after conception.

Sex ratio plays an important role in the maintenance of a species influencing the number and the most species give birth to equal numbers of male and female following the typical Mendelian sex ratio (1:1) (Leigh 1970). But some organisms show deviations from this typical sex ratio. This may be attributed to the fact that environmental factors influence the physiological state of reproducing animals (Anderson 1961, Charnov and Bull 1977). The environment influences the sex ratios in a large number of invertebrates. The role of infestation due to a pest is directly linked with its sex ratio and offspring production.

The haplodiploid sex determination system of most parasitoid wasps allows females to control the sex ratio (ratio of female progeny to total progeny), because they can control whether an egg is female (fertilized) or male (unfertilized). The topic of offspring of sex ratios in parasitoid wasps has been reviewed extensively by Flanders (1939, 1946), Kochetova (1977) and King (1987).

Clausen (1939) noted that of several factors that regulate the sex ratio in parasitic Hymenoptera. The two most important factors are the size and species of the host. King (1987) defines four factors that may influence offspring sex ratios in parasitoid wasps, parental characteristics, host characteristics, and factors influencing local mate competition. Charnov *et al.* (1981) reported that the sex of parasite offspring would vary with host size differentially and affects the fitness gains of males vs females. Female eggs should be gained more by developing in or on large hosts than males.

Most studied scored emergence sex ratio (i.e., the sex ratio at emergence from hosts on or in which eggs had been oviposited) and Charnov's (1982) careful review of the data emphasise the need to eliminate differential mortality of male and female offspring as a possible cause for the differences in sex ratio before equating the emergence sex ratio with the primary sex ratio (i.e., the sex ratio at oviposition).

The present experiment was undertaken to investigate whether host size have any influence on the sex ratio in *R. zae* and *H. sylvanidis* and *X. flavipes* respectively.

Materials and methods

The newly emerged adult parasitoids of *R. zae* and *H. sylvanidis* and predator *X. flavipes* were collected from the laboratory culture and each was transferred to different petri dishes (8.5 cm diam.) for mating. After mating the mated females were released individually in 20 previously reared 4th instar larvae and pupae of *T. castaneum* separately for egg laying. All the petri dishes were kept in the incubator.

Every 24 hours the parasitized larvae and pupae were replaced from the incubator and fresh larvae and pupae were supplied. As the adult parasitoids and predator are difficult to handle and examine, the ovipositing females were collected by an aspirator and kept in it. After the introduction of fresh larvae and pupae in place of parasitized ones, the parasitoids and predator were reintroduced in the petri dishes very quickly and again placed these in the incubator. These operations were repeated daily until all the female parasitoids and predator died. The parasitized larvae and pupae were reared until adult emergence. Total number of male and female were recorded. Data were obtained for twenty mated females of *R. zae*, *H. sylvanidis* and *X. flavipes* separately. Similar process was applied in case of *T. confusum*.

Results

The sex ratio of *R. zea*, *H. sylvanidis* and *X. flavipes* was highly female-biased and was influenced on the size of 4th instar larvae and pupae of *T. castaneum* and *T. confusum*. The proportion of female increased on 4th instar larvae. Sex ratios of the parasitoids and predator reared on 4th instar larvae and pupae. The total number of males and females of the parasitoids and predator, *R. zea*, *H. sylvanidis* and *X. flavipes* along with number of samples are given in Tables 7, 8, 9 and 10. It was observed that the calculated values were significant at 0.001 level of probability for all the above parasitoids and predator.

Table 7. Sex ratios of *R. zea*, *H. sylvanidis* and *X. flavipes* reared on 4th instar larvae of *T. castaneum*.

Name of the parasitoids/predator	No. of F ₁ progeny emerged	Progeny production		Percent of (%)		Sex ratio M:F	χ^2 values	P values
		Male	Female	Male	Female			
<i>R. zea</i>	80	15	65	18.75	81.25	1:4.33	39.06	P<0.001
<i>H. sylvanidis</i>	56	13	43	23.21	76.79	1:3.30	28.70	P<0.001
<i>X. flavipes</i>	140	22	118	15.71	84.29	1.5.36	47.03	P<0.001

Table 8. Sex ratios of *R. zea*, *H. sylvanidis* and *X. flavipes* reared on pupae of *T. castaneum*.

Name of the parasitoids/predator	No. of F ₁ progeny emerged	Progeny production		Percent (%)		Sex ratio M:F	χ^2 values	P values
		Male	Female	Male	Female			
<i>R. zea</i>	26	7	19	26.92	73.08	1:2.71	21.30	P<0.001
<i>H. sylvanidis</i>	23	6	17	26.09	73.91	1:2.83	22.86	P<0.001
<i>X. flavipes</i>	50	15	35	30.00	70.00	1:2.33	16.00	P<0.001

Table 9. Sex ratios of *R. zea*, *H. sylvanidis* and *X. flavipes* reared on 4th instar larvae of *T. confusum*.

Name of the parasitoids/predator	No. of F ₁ progeny emerged	Progeny production		Percent (%)		Sex ratio M:F	χ^2 values	P values
		Male	Female	Male	Female			
<i>R. zea</i>	76	15	61	19.74	80.26	1:4.06	36.62	P<0.001
<i>H. sylvanidis</i>	54	12	42	22.22	77.78	1:3.50	30.86	P<0.001
<i>X. flavipes</i>	135	19	116	14.07	85.93	1:6.10	51.63	P<0.001

Table 10. Sex ratios of *R. zea*, *H. sylvanidis* and *X. flavipes* reared on pupae of *T. confusum*.

Name of the parasitoids/predator	No. of F ₁ progeny emerged	Progeny production		Percent (%)		Sex ratio M:F	χ^2 values	P values
		Male	Female	Male	Female			
<i>R. zea</i>	24	7	17	29.17	70.83	1:2.42	17.35	P<0.001
<i>H. sylvanidis</i>	21	6	15	28.57	71.43	1:2.50	18.36	P<0.001
<i>X. flavipes</i>	48	14	34	29.17	70.83	1:2.42	17.35	P<0.001

Discussion

In hymenopterans parasitoids size and species of the host are the important factors that regulate the sex ratio (DeBach 1964, Wylie 1976, Hamilton 1967). Charnov (1982) discussed the causes of sex ratio change in parasitic wasps with host size; he admitted that most of the evidences for a change in the sex ratio with host size (or age) was data for the emerging sex ratio.

The results show that the numbers of females dominate over that of males in *R. zea*, *H. sylvanidis* and *X. flavipes*. The proportion of females increase on 4th instar larvae of *T. castaneum* and *T. confusum*.

The sex ratio of *R. zea* and *H. sylvanidis* are not well documented. But bethylid sex ratios are nearly always female biased, almost certainly due to moderate to high levels of sibling mating (Hardy and Mayhew 1998). As expected under sibling mating, bethylidae sex ratios generally have low variance, although deviations from binomial distribution are not in all cases significant (Hardy 1992, Hardy *et al.* 1998). The evidence for sex ratio variance in *C. hyalinipennis* is equivocal, treating offspring-batches as the sampling unit indicated overdispersion, while nonsignificant underdispersion was found when the sampling unit was restricted to offspring on individual hosts.

Perez-Lachaud and Hardy (2001) described alternate hosts or bethylid parasitoids of the coffee berry borer, *Hypothenemus hampei*. They found the overall sex ratio of this parasitoids in three different hosts were decreased significantly as brood size increased. According to Flanders (1946) and Doutt (1959), the spermatheca becomes a sex changing mechanism when it contains spermatozoa. During oviposition, spermathecal gland activated stimuli help to discharge sperms for fertilization.

Sary (1970) opined that sex ratio is determined through the peculiar mechanisms of the female's parasitoids, which responded to stimuli of extrinsic factors. On the contrary (Flanders 1939,1956) the sex ratio of arrhenotokous species is influenced both by intrinsic as well as extrinsic factors. Moreover, the sphincter muscles of the spermathecal duct regulate the movement of the sperms to fertilize the ovulated haploid egg and probably the behaviour of the sphincter muscle is externally initiated (Flanders 1969). However, it may be due to an instinctive behaviour of the parasitoids to produce male and female progeny by the same mated female in establishing its race.

According to White (1973), Mackaure (1976) and van den Assem (1977) natural selection favours a mechanism that ensures the production of at least some males in facultative arrhenotokous wasps. The experimental evidence of Mackaure (1976) though it was rejected by van den Assem (1977) who reported that the inhibition of

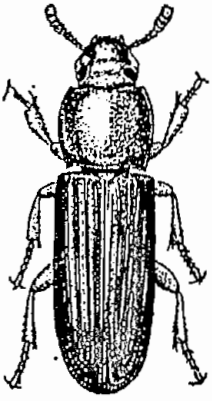
fertilization is a by-product inherent in the physiological of the reproductive system instead of a specific product of selection. Due to the insufficient supply of sperms received during copulation needed for fertilization of all the ovulated eggs.

Arbogast (1975) assumption that the sex ratio of *X. flavipes* was 1:1. In fact, the observed ratio of males to females did not differ significantly from unity at most of the temperatures and humidities. It was, however, greater than unity ($P=0.02$) at 20°C and 35% R.H. and less than unity ($P=0.04$) at 30 and 56% R.H. Sokal and Rohlf (1981) described the another anthocoridae bug *Lyctocoris campestris* deviation in sex ratio of emerging adults from 1:1 and differences in sex ratios of emerging adults at each temperature-relative humidity combination were tested using a G statistic. Parajulee *et al.* (1975) described the sex ratio (percentage of males) of emerging adult *L. campestris* ranged from 0.40 to 0.70 at different temperature-relative humidity combinations, but was not significantly different from 1:1 for any of the temperature or relative humidity regimes tested.

However, the result of the present investigation indicates that at least the following factors influence the sex ratio mechanism in the proposed area of study:

- i) high parasitic density
- ii) availability of their hosts
- iii) testing behaviour for site and instar before oviposition
- iv) higher percentage of successful matings
- v) lack of extreme temper condition during their occurrence.

So, the above factors would have complex effect on the preponda females; although further investigation is necessary to fill up the gap regarding the matter.



Chapter 10

Summary and conclusion

Summary and conclusion

Tenebrionide pests of stored products are widely distributed throughout the world. They infest stored products and frequently cause great damage and losses. Even a wide range of materials can be damaged or destroyed by infestation with Tenebrionides.

The red flour beetle *T. castaneum* and the confused flour beetle *T. confusum* causes serious damage and losses to grains, flour and mixed products particularly in the tropics. They are generally found in granaries, mills, warehouses, feeding on rice flour, wheat flour, suji, bleached and unbleached white flour, breakfast food, cornmeal, barley flour and oatmeal. The beetles are unable to feed on whole cereal grains because their mouthparts are not suitable for attacking large and hard pieces of food and thus, they have the status of secondary pest. Much of the damage done by *Tribolium* is directly to the kernels (germ and endosperm).

Infestations by Tenebrionides are usually controlled by treatments with insecticides. However, insecticides may cause hazards to man and the environment. Especially in the storage of small subsistence farmers in the tropics the use of insecticides may be dangerous and costs prohibitive. Hence, there is a need for the development of alternative methods such as biological control, an efficient component in Integrated Pest Management (IPM).

Two species of parasitoids, viz. *Rhabdopyris zae* (Waterston) and *Holepyris sylvanidis* (Brethes) (Hymenoptera: Bethyridae) and one species of predator were recorded from the stored wheat flour infested pest, *Tribolium*. Of these, *R. zae* and *H. sylvanidis* are the solitary and larval-pupal ectoparasitoids whereas *X. flavipes* is the predator. Biological parameters of all these species are described in considerable details.

Mating of *R. zae* and *H. sylvanidis* took place immediately after the emergence of the adults. This was an elaborate and complex process. The male took an active role in

mating. His overtures to the female and expressed in a series of movements, including chasing of the female, sudden stopping and wobbling from side to side, fluttering of the wing, and contacting the female with outstretched antennae. Males are polygamous but females are monogamous.

Eighteen discrete ovipositional behaviours were shown by *R. zae* and *H. sylvanidis*. The behaviours were categories as: search, move away, bite, antennal tip, antennal side, head/mouth, clin on, sting, turn, pause, attempted bite, groom, catatonic, chew, pull, rub, touch and rest. Oviposition more frequently occurs on 3rd instar larval stage. No oviposition took place on the egg of *Tribolium*. The deposited egg is of the typical hymenopteriform. There are three larval instars and the larvae are hymenopteriform. The last instar forms an inactive or motionless pre-pupae. The pupa is exarate type. The imago emerges by cutting a small circular hole on the upper portion of the cocoon. Only one adult parasitoid emerges from a single cocoon, indicating that the parasitoids are solitary.

The developmental periods differed in the male and female of *R. zae* and *H. sylvanidis*. For the male, it took 19.06 ± 0.59 days and 15.50 ± 0.62 days when *T. castaneum* was supplied as host but 20.10 ± 0.77 days and 16.00 ± 0.47 days when *T. confusum* was supplied as host to complete the life-cycle at $30 \pm 0.5^\circ\text{C}$ and $70 \pm 0.5\%$ R. H. Both the parasitoids require host fluids for greater longevity and there was a significant difference in longevity between the sexes ($P < 0.001$). The mated females always produced higher number of eggs when fed host fluids in both *R. zae* and *H. sylvanidis*. The sex ratio of the parasitoids was highly female biased.

Mating in *X. flavipes* occurs when the females sexually mature. The deposited egg is elongated-oval and blackish with yellow spots. The nymphs are shining and sparsely setose. The tarsi are 2-segmented. There are four pairs of dorsal abdominal scent gland. There were five nymphal instars in the life of *X. flavipes*. The body of the first instar is pale brownish-yellow. The eggs are deep brick red. Wing pads are lacking. In the

second instar wing pads barely visible. Ocelli appear as small red spots and anterior portion of head and thorax infuscata in the third instar larva. In the fourth instar eyes deep brick red and wing pads extending to abdominal segment. The fifth instar is dark brownish yellow to brown with pale brownish yellow legs, antennae and rostrum. The develop wing pads provides the most reliable method of separating instars 2-5.

The male and female *X. flavipes* can be easily distinguished by the shape of the abdomen, which is bilaterally symmetrical in females. Brachypterous and macropterous forms occur in both the sexes. Developmental time from oviposition to adult emergence, male and female was differ. In male, it took 16.99 ± 1.86 days and 17.80 ± 1.30 days on *T. castaneum* and in female 16.00 ± 1.63 days and 17.00 ± 1.63 days on *T. confusum* respectively at $30 \pm 0.5^\circ\text{C}$ and $70 \pm 0.5\%$ R. H. The mean female adult longevity was highest when both *T. castaneum* and *T. confusum* was supplied continuously. A mated female produced greater number of eggs when host fluids continuously supplied. The sex-ratio was always female biased.

The status of biological control as a viable pest control strategy has increased dramatically in recent years. It is unnecessary to argue divisibly whether chemical or biological control rules the roost. What seems vital today is that both man and environment remain safe without prejudice to plant protection and animal health. There is no doubt that the amount of research on biological control will increase many-fold in the future. Even better use of bio-control agents will be possible in future, when information and experience are gained.

The parasitoid wasps *R. zea*, *H. sylvanidis* and *X. flavipes* are less known and studied. Their biology does not depart from that of other related parasitoids. However, due to the particular status of their host, they appear to have a promising future in research.

The parasitoids and predator of stored-product insect pests are unique in several features that it remains in abundance throughout the year even when the host are sparse although exhibit fluctuation, which appears to be correlated with the abundance of the

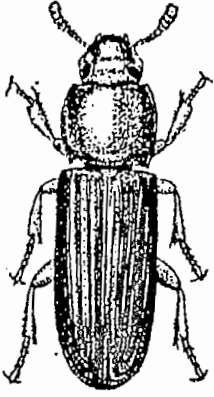
host. They meet salt's interpretations of ecological, psychological and physiological selection which eventually represent DeBach's (1964) interpretation of host habitat finding, host finding, host acceptance and host suitability.

It is found that the multiplications of *R. zaeae*, *H. sylvanidis* and *X. flavipes* are fairly short and presumably has a number of generations each year. This is an advantage on the part of the parasitoids and predator since in case of *Tribolium*. There is no definite breeding season and throughout the year larvae and early pupae of *Tribolium* could be available. Hence the parasitoids have no scarcity of host larvae and pupae for oviposition and multiplication.

R. zaeae and *H. sylvanidis* are similar in their biological attributes. Their life cycles are of very short duration compared to other hymenopteran as well as bethylid parasitoids. They have high fecundity, and a single female can produce an average of about 104.05 ± 1.79 and 78.30 ± 1.65 eggs in their lifetime. They can easily be reared on larvae and pupae of *Tribolium*. The mature larvae and early pupae are most suitable for egg deposition, it is suggested that for mass rearing of the parasitoid in biological control programmes these stages of *Tribolium* can be utilized.

Like *R. zaeae* and *H. sylvanidis*, *X. flavipes* is unique in several features. Its life cycle also is of very short duration. It has a high fecundity, and a single female devours an average of 37.3 prey larvae of *Tribolium* to complete life cycle. They can easily be reared in *Tribolium* although they have many other hosts. Since the early stage of *Tribolium* is most suitable, it is suggested that for mass rearing of *X. flavipes* in biological control programmes this larval stage be utilized.

Moreover, it could be concluded that pest suppressing value of *R. zaeae*, *H. sylvanidis* and *X. flavipes* is enhanced by parasitizing and devouring of larvae and early pupae of *Tribolium*. Judging from the fact that all the life-stages of *Tribolium* occur together in the natural condition it seems that the above behaviour of the parasitoids and predator helps in effecting a higher percentage of control of the pest by parasitizing the stages concurrently.



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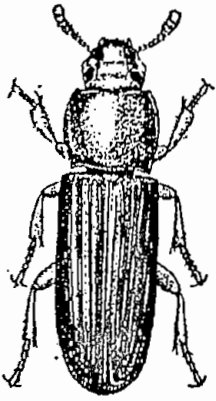
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Appendices

Appendices

Appendix Table 1. Duration of immature stages of *R. zeae* of *T. castaneum*.

Stages	Duration (days)		
	N	Range	Mean±SE
Incubation period	20	1.50-1.80	1.63±0.07
Larva			
1 st instar	20	1.60-2.00	1.80±0.09
2 nd instar	20	1.30-1.80	1.53±0.11
3 rd instar	20	1.50-1.80	1.63±0.07
Pre-pupa	20	3-4	3.33±0.27
Pupa	20	9-10	9.66 ±0.27
Male	20	17.90-20.04	19.06±0.59
Female	20	18.90-21.40	20.14±0.58

Appendix Table 2. Duration of immature stages of *R. zeae* of *T. confusum*.

Stages	Duration (days)		
	N	Range	Mean±SE
Incubation period	20	1.70-1.90	1.81±0.04
Larva			
1 st instar	20	1.70-2.00	1.85±0.07
2 nd instar	20	1.40-1.90	1.77±0.09
3 rd instar	20	1.70-2.00	1.83±0.07
Pre-pupa	20	3.50-4.00	3.05±0.55
Pupa	20	9.50-1.00	10.16 ±0.36
Male	20	18.50-21.80	20.1±0.77
Female	20	19.00-22.80	21.2±0.92

Appendix Table 3. Duration of immature stages of *H. sylvanidis* of *T. castaneum*.

Stages	Duration (days)		
	N	Range	Mean±SE
Incubation period	20	1.50-2.00	1.76±0.11
Larva			
1 st instar	20	2.00-2.50	2.33±0.13
2 nd instar	20	1.50-2.00	1.76±0.11
3 rd instar	20	1.50-2.00	1.76±0.11
Pre-pupa	20	1.00-1.50	1.30±0.12
Pupa	20	7.00-8.00	7.33±0.27
Male	20	14.50-17.00	15.50±0.62
Female	20	15.00-18.00	16.66±0.72

Appendix Table 4. Duration of immature stages of *H. sylvanidis* of *T. confusum*.

Stages	Duration (days)		
	N	Range	Mean±SE
Incubation period	20	1.60-2.10	1.83±0.11
Larva			
1 st instar	20	2.20-2.70	2.46±0.12
2 nd instar	20	1.60-2.00	1.76±0.09
3 rd instar	20	1.60-2.10	1.90±0.12
Pre-pupa	20	1.20-1.60	1.66±0.17
Pupa	20	7.20-8.50	7.63±0.35
Male	20	15.00-17.00	16.00±0.47
Female	20	15.50-18.70	17.15±0.80

Appendix Table 5. Duration of immature stages of *X. flavipes* of *T. castaneum*.

Stages	Duration (days)		
	N	Range	Mean±SE
Incubation period	20	4-5	4.22±0.69
1 st nymph	20	2-3	2.53 ±0.23
2 nd nymph	20	2-3	2.46±0.21
3 rd nymph	20	2-3	2.66 ±0.27
4 th nymph	20	2-3	2.38 ±0.18
5 th nymph	20	2-4	3.00±0.47
Male	20	15-21	16.99±1.86
Female	20	14-20	16.00±1.63

Appendix Table 6. Duration of immature stages of *X. flavipes* of *T. confusum*

Stages	Duration (days)		
	N	Range	Mean±SE
Incubation period	20	4.50-5.00	4.70±0.12
1 st nymph	20	2.10-3.20	2.76±0.28
2 nd nymph	20	2.00-3.10	2.75±0.30
3 rd nymph	20	2.50-3.00	2.83±0.28
4 th nymph	20	2.60-3.00	2.98±0.23
5 th nymph	20	2.50-4.00	3.16±0.36
Male	20	16.00-21.00	17.80±1.30
Female	20	15.00-20.00	17.00±1.63

Appendix Table 7. Effect of different types food on adult longevity of *R. zea* (N=20).

Foods	Longevity (days)			
	Range		Mean±SE	
	Male	Female	Male	Female
Honey	6-15	8-30	10.6±0.58	19.7±1.49
Glucose	6-12	9-20	8.75±0.42	12.85±1.04
Sucrose	5-10	6-10	6.25±0.30	8.4±0.33
Distilled water	5-9	6-14	6.05±0.28	10.33±0.55
<i>T. castaneum</i>	6-14	18-22	10.85±0.48	19.85±0.33
<i>T. confusum</i>	4-10	14-20	9.85±0.50	19.20±0.56
Control	2-3	4-6	2.35±0.11	5.10±0.18

ANOVA

Sources of variation	Degree of freedom	Sum of squares	Mean squares	F-values
Observation(0)	19	260.92857	13.73308	1.89 *
Treatment	13	8389.27143	645.32857	88.81**
Sex (S)	1	3155.71429	3155.71429	434.27 **
Food (F)	6	4060.52143	676.75357	93.13**
S×F	6	1173.03571	195.50595	26.90 **
Error	247	1794.87143	7.26669	

cv=26.1%

**=Significant at 1% level; *= Significant at 5% level

Appendix Table 8. Effect of different types food on adult longevity of *H. sylvanidis* (N=20).

Foods	Longevity (days)			
	Range		Mean±SE	
	Male	Female	Male	Female
Honey	5-14	8-28	7.24±0.65	16.7±1.41
Glucose	5-13	7-22	7.20±0.47	14.95±1.19
Sucrose	4-10	7-16	6.15±0.45	11.25±0.73
Distilled water	4-9	6-14	5.75±0.31	10.0±0.51
<i>T. castaneum</i>	5-12	16-20	7.9±0.57	18.10±0.35
<i>T. confusum</i>	5-12	17-21	7.00±0.42	17.2±0.37
Control	1-3	2-5	1.85±0.15	2.95±0.20

ANOVA

Sources of variation	Degree of freedom	Sum of squares	Mean squares	F-values
Observation(0)	19	260.6285571	13.717293	1.73 *
Treatment	13	7246.842857	557.449451	70.15**
Sex (S)	1	3250.414286	3250.414286	409.02 **
Food(F)	6	3284.492857	547.415476	68.88 **
SXF	6	711.935714	118.655952	14.93 **
Error	247	1962.871429	7.946848	

cv=29.3%

**=Significant at 1% level; *= Significant at 5% level

Appendix Table 9. Effect of different types food on adult longevity of *X. flavipes* (N=20).

Foods	Longevity (days)			
	Range		Mean±SE	
	Male	Female	Male	Female
Honey	4-15	20-26	8.66±0.88	24.1±0.34
Glucose	5-15	17-25	7.45±0.72	20.13±0.45
Sucrose	4-14	15-21	6.90±0.8	18.95±0.64
Distilled Water	2-12	15-21	6.35±0.47	17.5±0.39
<i>T. castaneum</i>	4-14	30-45	7.05±0.64	37.2±0.74
<i>T. confusum</i>	4-12	30-43	6.25±0.50	36.15±0.56
Control	3-8	6-15	5.15±0.3	9.55±0.56

ANOVA

Source of variation	Degree of freedom	Sum of squares	Mean squares	F-values
Observation (0)	19	188.22857	9.90677	1.35 ns
Treatment	13	31606.58571	2431.27582	331.00 **
Sex (S)	1	19455.55714	19455.55714	2648.73 **
Food (F)	6	6485.03571	1080.83929	147.15 **
SXF	6	5665.99286	944.33214	128.56 **
Error	247	1814.27143	7.34523	

cv=17.9%

**=Significant at 1% level; ns= not significant.

Appendix Table 10. Effect of different types of food on the production eggs of *R. zea*.

Food	Fecundity	
	Range	Mean \pm SE
Honey	65-97	80.95 \pm 2.04
Glucose	55-68	63.35 \pm 0.92
Sucrose	46-55	50.35 \pm 0.63
Dist. Water	40-56	49.30 \pm 1.27
<i>T. castaneum</i>	88-115	104.05 \pm 1.79
<i>T. confusum</i>	88-113	102.55 \pm 1.74
Control	28-50	39.05 \pm 1.56

ANOVA

Sources of variation	Degree of freedom	Sum of squares	Mean squares	F-values
Observation (0)	19	844.93571	44.47030	1.02 ns
Food (F)	6	86643.60000	14440.60000	330.30**
Error	114	4984.11429	43.72030	

cv=9.5%

**=Significant at 1% level; ns=not significant

Appendix Table 11. Effect of different types of food on the production eggs of *H. sylvanidis*.

Food	Fecundity	
	Range	Mean \pm SE
Honey	48-76	61.20 \pm 1.79
Glucose	40-56	48.20 \pm 1.04
Sucrose	32-44	38.45 \pm 0.76
Dist. Water	32-48	40.25 \pm 1.03
<i>T. castaneum</i>	64-88	78.30 \pm 1.64
<i>T. confusum</i>	64-89	76.68 \pm 1.65
Control	28-48	34.30 \pm 1.35

ANOVA

Source of variation	Degree of freedom	Sum of squares	Mean squares	F-values
Observation (0)	19	658.54286	34.66015	<1
Food (F)	6	40492.07148	6748.67857	173.15**
Error	114	4443.35714	38.97682	

cv=11.6%

**=Significant at 1% level

Appendix Table 12. Effect of different types of food on the production eggs of *X. flavipes*.

Food	Fecundity	
	Range	Mean \pm SE
Honey	66-84	76.20 \pm 1.18
Glucose	60-70	65.20 \pm 0.77
Sucrose	40-60	50.20 \pm 1.40
Dist. Water	38-60	47.40 \pm 1.43
<i>T. castaneum</i>	88-116	98.65 \pm 1.81
<i>T. confusum</i>	86-115	97.05 \pm 1.73
Control	28-50	42.22 \pm 1.47

ANOVA

Source of variation	Degree of freedom	Sum of squares	Mean squares	F-values
Observation (0)	19	1551.45714	81.65564	2.27**
Food (F)	6	65931.04286	10988.50714	304.85**
Error	114	4109.24286	36.04599	

cv=8.8%

**=Significant at 1% level

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