Materials and Methods
CHAPTER III

MATERIALS AND METHODS

The present investigations were carried out in the farmers' field of Barpeta district and laboratory of Department of Zoology, Gauhati University during 1998-2001. The Barpeta district is situated in the Lower Brahmaputra Valley Zone of Assam (Fig. 1) between 26°5' N and 26°51' N latitudes and 90°38' E and 91°20' E longitudes. This investigation covered 10 major rice growing pockets of Barpeta district namely, Pathsala, Bhawanipur, Howly, Sorbhog, Mandia, Keotkuchi, Patbaushi, Bhella, Jania and Kayakuchi.

3.1 Sources of infestation of *Sitophilus oryzae* Linn. and *Sitotroga cerealella* Oliv.

3.1.1 Pre-harvest infestation

Investigations on pre-harvest infestation of rice grains by *S. oryzae* and *S. cerealella* were done by collecting field samples during summer and winter season cultivation of 1999 in rice fields of Pathsala, Bhawanipur, Howly, Sorbhog, Mandia, Keotkuchi, Patbaushi, Bhella, Jania and Kayakuchi area of Barpeta district. At each location, field sample collection were made at a distance of 50, 100, 200, 300, 400, 500, 600, 700 and 800 meters away from farmers' storage precints. Two sets of samples were drawn, one during June-July for cultivar 'Hasakumra' (summer rice) and another during November-December for cultivar 'Aijong' (winter rice) which are most popular and commonly cultivated cultivars in this district.
Fig. 1. Map of Barpeta district showing 10 major rice growing pockets.
Standing spikelets from each marked spots were randomly collected five days before harvest. In each sample 120 spikelets were picked up in polythene bags, properly tied with rubber band, labelled them spotwise and brought to the laboratory for infestation estimation. The collected spikelets were then divided into three sub-samples each having 40 spikelets and were kept in glass jars covered with muslin cloth and properly tied with rubber bands. The sample jars were kept for 40 days under laboratory conditions for emergence of the adults. The populations of emerged adults of *S. oryzae* and *S. cerealella* were counted, and the percentage of infestation per 500 g sample by both the species were calculated.

### 3.1.2 Grain residues and debris

The presence of adult *S. oryzae* and *S. cerealella* in residues and debris of two types of storage receptacles, *viz.*, 'Gutibhoral' and 'Duli' were estimated following the methods of Coombs and Freeman (1955). The presence of both these pest species in grain residues was examined in randomly selected crevices of the wall and floor of receptacles of the 10 selected rice growing pockets of Barpeta district during the period 1999 to 2000. The insects found, other than *S. oryzae* and *S. cerealella* were excluded from the purview of present investigation. To maximise the chances of appearance of the pest species in storage receptacles, 10 numbers each of 4 year old 'Gutibhorals' and 'Dulis' were randomly selected for collecting samples. The floors and walls of each empty receptacle were carefully brushed and samples of 500 g residues were collected from each and examined in the laboratory for the presence of these two insects.
Similarly, samples of 500 g thrown out debris were also collected from the immediate vicinity of each storage receptacles during the Summer and Autumn seasons of 1999 and 2000. The samples were examined in laboratory for the presence of the two insect-pests in debris.

Appearance of any number of adults of *S. oryzae* and *S. cerealella* found in grain residues and debris were recorded. The number of adult appearance of each species were also computed and recorded.

### 3.1.3 Other plants in field vicinity

In order to find out the existence of sheltering plants / alternate hosts under field conditions, the naturally growing plants in and around the experimental rice fields of Barpeta district were examined *in situ* to ascertain the presence of *S. oryzae* and *S. cerealella*. Seeds and earheads of these plants were collected and kept in the laboratory for 45 days for possible emergence of *S. oryzae* and *S. cerealella*. Also to test the possibility of these plants acting as alternate hosts for these two insects pests, artificial inoculations were made in the laboratory by releasing adults of *S. oryzae* and eggs of *S. cerealella* on the kernels and seeds of these 10 plant species, namely, Fox tail grass (*Setaria glauca*), Wild rice (*Oryza perennis* and *O. fatua*), Red root pig weed (*Amaranthus retroflexus*), Weed plants (*Chrysopogon montanus*; *Cyperus rotundus*; *Echinochloa crussgalli* and *E. colonum*) 'Kahua' (*Saccharum spontaneum*) and Morning glory (*Ipomoea reptans*).

Seeds were collected (20 g) from each of these plant species and exposed separately in the laboratory to *S. cerealella* by placing 100 eggs in a desiccator with their mouth covered with a muslin cloth. Eggs of the *S. cerealella* were obtained following the method of Sundararajan (1978).
The desiccator was then kept in an incubator at 30°C and 70% relative humidity for 45 days for hatching and development of the larvae, if any on the seeds of each plant species. Observations were noted respective to these plant species.

Similar artificial inoculations were also carried out with *S. oryzae*. Ten pairs of freshly emerged (mated) adults of *S. oryzae* were released on 20 g seeds of each plant species kept in desiccators and allowed to develop at normal room temperature (24-32°C) and relative humidity (65-75%) for 45 days. Observations were recorded for each of these plant species exposed.

### 3.1.4 Crevices of tree barks

Pieces of wood (6 cm x 6 cm) with barks were cut carefully from seven tree species namely, datepalm or 'khejur' (*Phoenix sylvestris*), Indian palm or 'bagari' (*Zizyphus jujuba*), drumstick or 'sajina' (*Moringa oleifera*), yellow oleander or 'karabi' (*Thevetia peruviana*), peacock flower or 'radhachura' (*Caesalpinia pulcherrima*), gold mohur or 'krishnachura' (*Delonix regia*) and mango (*Mangifera indica*) that were grown around the rice fields and were observed the presence of *S. oryzae* and *S. cerealella*. After thorough examination the pieces were kept separately in jars (16x12x10 cm) in which five pairs of *S. oryzae* (mated) were released. The jars were covered with muslin cloth taking care so that insects could not escape. Four such replications were maintained for the experiment. A similar experiment with four replications were carried out in the laboratory with five pairs of *S. cerealella* (mated). The experiments were maintained in room temperature for 45 days for emergence of adults. The investigation was done during summer and winter of 1999 and 2000.
3.2 Distribution of *S. oryzae* and *S. cerealella* in commonly used storage receptacles in Barpeta district

3.2.1 Description of storage receptacles

The commonly used storage receptacles in Barpeta district have been briefly described below.

3.2.1.1 'Gutibhoral'

It is an outdoor traditional farm level storage receptacle for bulk storage of threshed paddy grains of Assam, constructed on a raised platform with wooden or concrete pillars. The four walls of the storage structure is made up of strip bamboo and wooden poles. Inner sides and floors are plastered with mud. The roof is generally covered with G.I. sheet or asbestos sheets or thatch. A window like opening serves the purpose of both inlet and outlet. The size of the structure varies according to the quantity of rice grains stored (Plate 1).

3.2.1.2 'Duli'

'Duli' is indigenous storage structure of Assam. It is a sort of big bamboo basket made up of fine strips of bamboo and is round in shape. It is 75 cm to 1 meter in diameter and 1.5 to 2 meters in height. The inner sides are plastered with a mixture of mud and cowdung. Storage capacity varies from 2 - 10 quintals depending upon its size (Plate 1).

3.2.1.3 'Tom'

'Tom' is a special type of storage receptacle used at farmers' level in Assam and is exclusively used for storage of paddy seeds. It is made up of very fine strips of bamboo, round in shape with big holes. Then a thick lining of dry paddy straw is lined around the inner side to hold the grains intact. After filling this receptacle with rice seeds its open side is twisted and tied with its fine bamboo strips. Storage capacity is about 10-20 kg. It is very suitable for storage of paddy
seed in humid climate of Assam, as the germination percentage of paddy stored in 'tom' was found to be 95-100% after 9 months of storage (Plate I).

3.2.1.4 Hessian cloth bag

It is jute bag of standard size for storage and transportation of food grains. The grains are properly dried before packing, stitched and kept inside the room on a wooden plank or bamboo dunnage (Plate I).

3.2.1.5 Polythene bag

Polythene bags of various thickness are used by some farmers for storing paddy. The paddy so kept in the bags are made airtight by wrapping very tightly with ropes. The bags are then either kept in bamboo machang or in racks (Plate I).

3.2.1.6 Plastic bin

It is a low-cost indoor bin made up of plastic. Being airtight plastic bins of various sizes are also used for storing rice grains by many farmers (Plate I).

3.2.1.7 Earthen pot

It is a small earthen storage structure occasionally used by the farmers for storing rice / paddy. It is made up of burned clay and covered. Storage capacity varies from 2-30 kg depending upon its size. The structure was filled up with paddy and placed on a raised platform (Plate I).

3.2.1.8 Metallic bin

It is made up of 24 Gauze galvanised sheet, cylindrical, reasonably airtight with cover at the top and an outlet at the bottom. Metallic bins were developed by Indian grain storage Institute, Hapur and used by many farmers for storage of rice grains (Plate I).
Plate I

Plate I. Different types of Storage Receptacle used at farmers' level
3.2.2 Percentage of stored paddy in different types of receptacle at farmers' level in Barpeta district

To assess the farmers' relative preference of different storage receptacles, a survey of 10 major rice growing pockets of Barpeta district was conducted during 1999-2000. The samples of 20 farmers' homestead from each of the rice growing pockets, adopting various cultural practices were selected on the basis of land holdings. In order to elicit the information from the respondents, a questionnaire was prepared and it was pre-tested for its completeness and accuracy on a sample of 20 respondents of a non-sampled village. The information regarding type of storage receptacles most commonly used by them and its relevant aspects were collected from the farmers. The total quantity of threshed rice stored and the storage period in different receptacles, viz., 'Gutibhoral', 'Duli', 'Tom', hessian cloth bag, polythene bag, plastic bin and earthen pot were also ascertained. The percentages of quantity of paddy stored in different receptacles were then estimated.

3.2.3 Adult population of \textit{S. oryzae} and \textit{S. cerealella} in different receptacles

Ten major rice growing pockets of Barpeta district, namely Pathsala, Bhawanipur, Howly, Sorbhog, Mandia, Keotkuchi, Patbaushi, Bhella, Jania and Kayakuchi were selected for this study (Fig. 1).

Seven types of storage receptacle which are most commonly used by the farmers were selected from each rice growing pocket of the 10 study areas. The samples of rice grains, each weighing 500 g were taken from four points of each storage receptacle which were atleast six months in storage. Two such samples were drawn from each storage receptacle. The collected samples were then taken in polythene bags, tied with rubber band, labelled
and kept in the laboratory at room temperature and humidity for estimation of adult *S. oryzae* and *S. cerealella* populations. The number of emerged adults were counted and the mean population was calculated. The investigation was conducted during 1999 and 2000 storage season.

### 3.3 Storage practice at farmers' level under agro-climatic conditions of Barpeta district

In order to assess the adoption percentage of storage practices by the farmers of Barpeta district, a survey work in the selected 10 major rice growing pockets of the district was conducted during the post harvest periods of 1999 and 2000. In this investigation, 10 farmers in each of the 10 rice growing pockets as stated elsewhere were selected. The different storage practices adopted by these farmers to store their total quantity of both threshed and unthreshed grains were assessed. The percentages of different practices adopted by the farmers were then worked out on the basis of survey results.

### 3.4 Effect of storage practices on infestation of *S. oryzae* and *S. cerealella*

#### 3.4.1 Maintenance of stock culture

The stock culture of *S. oryzae* and *S. cerealella* were obtained from farmers' receptacles of Barpeta district. *S. oryzae* and *S. cerealella* were reared in polished rice and cleaned rice (husked rice) respectively, of the cultivar 'Aijong'. Both the insect species were reared for three successive generations before the experimental work began (Plate II). Stock cultures of the insects were kept at room temperature and humidity. Fresh cultures of each species were set up every week by transferring 100-120 newly emerged adults into jars (30 x 17 x 9 cm) containing 250 g polished rice (for *S. oryzae*) and 250 g husked rice (for *S. cerealella*). Adults of *S. oryzae* were removed from culture by sieving...
Plate II

Culture of *S. oryzae*  
Culture of *S. cerealella*

Rice grains infested by *S. oryzae*  
Rice seeds infested by *S. oryzae*

Rice seeds infested by *S. cerealella*

Plate II. Culture of *S. oryzae* and *S. cerealella*, Rice grains and seeds infested by *S. oryzae* and *S. cerealella*
after three weeks of infestation. *S. cerealella* were separated using an aspirator. The moisture content of polished rice used for stock cultures of *S. oryzae* was maintained at 10 per cent level and that of rice grains for stock cultures of *S. cerealella* were adjusted at 12 per cent as suggested by Bordoloi (1990).

**3.4.2 Grain moisture content**

Grain moisture content was measured with the help of a OSAW Universal Moisture Meter.

**3.4.3 Infestation percentage**

The grains with germ points partially or wholly eaten up with exit holes of insect or with the endosperm otherwise eaten up externally were taken as damaged grains and all calculations for percentage of infestation or damage was based on those symptoms. The grains were spread over a thick mirror to facilitate quick detection of bored grains without turning the grain on its side. The total number of grains and the total number of weeviled and germ eaten grains in 500 g sample were counted and the infestation percentage was calculated using the formula

\[
\text{Infestation percentage} = \frac{\text{Total number of weeviled and germ eaten grains in 500 g sample}}{\text{Total number of grains in 500 g sample}} \times 100
\]

**3.4.4 Sexing**

**3.4.4.1 Sexing of *S. oryzae***

Sexing of *S. oryzae* was carried out by examining the dorsal surface of the Rostrum (snout) which is much more closely and strongly punctured in the male than in the female as per methodology of Richards (1947) and Halstead (1962).
3.4.4.2 Sexing of *S. cerealella*

Abdominal characteristics inspection as adopted by Prakash and Rao (1985) was used to separate the sexes of the *S. cerealella*. In the male the abdomen is thinner, pointed and blackish when viewed from ventral side. In the female the abdomen is bulky and long without any blackish colouration.

3.4.5 Grain sampling

Grain sampling was done by taking 500 g grains from four points of the storage receptacle and four such samples were drawn to make one composite sample.

3.4.6 Detection of hidden infestation

The grain samples were subjected to acid fuchsin staining test (Frankenfeld, 1948) for detection of hidden infestation if any. The egg masses of the insect on grains will be gelatinous deep cherry colour and infested seeds will be deep cherry red colour.

3.4.7 Storage practices

Infestation of *S. oryzae* and *S. cerealella* at three initial grain moisture contents (11%, 14% and 17%) and at three different periods of storage (3, 6 and 9 months) under three different storage practices (threshed clean, threshed unclean and unthreshed grains) adopted by the farmers of Barpeta district was considered to study the effect of storage practice, grain moisture content and period of storage on the infestation of *S. oryzae* and *S. cerealella* during 1998-2000.

3.4.7.1 Threshed clean grains

The investigation was carried out on 100 kg threshed and clean rice grains in the laboratory under ventilated condition of storage
in a commonly used receptacle 'Duli'. The rice grain used in this study was of the cultivar 'Aijong', that were free from any chemical treatment. After harvest of the crop, the grains were sieved and the dusts and other foreign matters were removed. The cleaned grains were then sundried for varying periods and the moisture content was brought to 11±0.2, 14±0.2 and 17±0.2 per cent. The grain samples were then subjected to acid fuchsin test (Frankenfeld, 1948) to detect if there was any hidden infestation. The infestation free grains were then transferred to storage receptacle, 'Duli'. Twenty five pairs of adult _S. oryzae_ and _S. cerealella_ were released into the receptacle. The receptacle was then covered with muslin cloth and tied all-around with a ribbon so that the insect could not escape from the structure. The percentage of grain infestation (mean) due to combined attack of _S. oryzae_ and _S. cerealella_ was recorded after 3, 6 and 9 months of storage. The experiment was conducted in 3 replications from November to August in 1998-99 and 1999-2000.

**3.4.7.2 Threshed unclean grains**

The rice grains of cultivar 'Aijong' were obtained from the farmers' threshing yards in unclean condition. The moisture contents of the grains were brought to 11±0.2, 14±0.2 and 17±0.2 per cent by drying in sun for varying periods. The procedure for conducting the experiment was similar to threshed clean grains described elsewhere. The experiment was conducted from October to July in 1998-99 and 1999-2000.

**3.4.7.3 Unthreshed grains**

In unthreshed storage practice the experiment was carried out with bundles of rice cultivar 'Aijong'. The crop for this particular
experiment was grown separately in cement pots. The crop grown in the pots were covered with fine cloth netting during vegetative and reproductive stages to prevent the field infestation of *S. oryzae* and *S. cerealella*. Immediately after harvest, the unthreshed bundles were sundried in batches for varying periods to bring the grain moisture level to 11±0.2, 14±0.2 and 17±0.2 per cent. Two bundles of unthreshed grains were then put into storage receptacle, 'Duli'. Twenty five pairs of adult *S. oryzae* and *S. cerealella* were released over the unthreshed bundles covered with muslin cloth and tied all-around to prevent escape of the insects. Sampling of grains was done at 3, 6 and 9 months after storage. Grain sampling from unthreshed bundles was done by taking fragmented bundles from four points and were immediately threshed to get the grains for determination of infestation percentage and moisture content. The experiment was conducted in 3 replications from November to August in 1998-99 and 1999-2000.

The data were subjected to analysis of variance to estimate the effects of various storage parameters on infestation.

### 3.5 Effect of storage conditions on infestation of *S. oryzae* and *S. cerealella*

Infestation of *S. oryzae* and *S. cerealella* at three initial grain moisture contents (11 %, 14 % and 17 %) and at three different storage periods (3, 6 and 9 months) under commonly practised storage conditions namely, airtight and ventilated conditions at farmers' level in Barpeta district was undertaken to study the effect of storage conditions on infestation of both the insect species.
The experiment was carried out during 1998-2000 harvesting season by storing rice grains in three airtight (metallic bin, plastic bin and polythene bag) and three ventilated (hessian cloth bag, 'Duli' and earthen pot) storage receptacles. Rice cultivar 'Aijong' was used in this experiment. After harvest of the crop, the grains were threshed and the dusts and other foreign matters were removed. The cleaned grains were then sundried in batches for varying periods to bring down the moisture level to 11±0.2, 14±0.2 and 17±0.2 per cent. The grains were free from any chemical treatment prior to or during the experimental storage. These grains were transferred to each of the receptacles and were subjected to natural infestation of *S. oryzae* and *S. cerealella*. Sampling was done at three different moisture contents and three different periods of storage to determine the infestation percentage with respective storage period and moisture content of grains. The experiment was conducted in 3 replications from November to August 1998-99 and 1999-2000.

The data were subjected to analysis of variance to estimate the effects of the experimental parameters on infestation.

### 3.6 Seasonal incidence of *S. oryzae* and *S. cerealella*

The seasonal incidence of *S. oryzae* and *S. cerealella* was studied by counting their populations in samples of grain collected from the most commonly used storage structure 'Gutibhoral'. The study was conducted during 1998-99 and 1999-2000. The cultivar 'Aijong' was stored in each 'Gutibhoral'. Four such replications were maintained for this investigation. The stored rice grains were under natural exposure to the attack of *S. oryzae*
and *S. cerealella* as both the insect species are dominant in the storage environment under the agro-climatic conditions of Barpeta district. About 500 g stored grains from each 'Gutibhoral' in respect of each replication was sampled in the middle of each month and kept in polythene bags and labelled.

The polythene bags were then brought to the laboratory and the sampled grains were kept in glass jars (30 x 17 x 9 cm) at room temperature and humidity to facilitate observations on the emergence of adults of *S. oryzae* and *S. cerealella*. Grains were observed daily for emergence of adults upto a period of one month. The total number of adults emerged of each species were recorded. All observations were made from 4 samples of 500 g grains each of the replication. Based on the total number of *S. oryzae* and *S. cerealella* emerged, the mean numbers of *S. oryzae* and *S. cerealella* per sample were computed. The temperature and humidity for each month were recorded to work-out the seasonal gradient. The population patterns of *S. oryzae* and *S. cerealella* as influenced by seasonal changes of ambient temperature (minimum temperature, maximum temperature) and relative humidity were worked out. The seasonal populations of both the insects were correlated with maximum temperature, minimum temperature and relative humidity; and the respective regression equations were found out.

### 3.7 Infestation of *S. oryzae* and *S. cerealella* in relation to physical characters and chemical constituents of grains

#### 3.7.1 Infestation of *S. oryzae* and *S. cerealella*

In order to assess the grain damage by each of the insect species, healthy and infestation free rice grains of 12 popular rice cultivars of 10 major rice growing pockets of Barpeta district were selected.
The cultivars tested in this experiment were 'Moinagiri', 'Balam', 'Baismati', 'Ranjit', 'Joha', 'Pankaj', 'Kolajoha', 'Aijong', 'Bora', 'Diga' (Bao), 'Katisali' and Hasakumra' (Plate III & IV). The grains of each rice cultivar, after sundrying was kept in desiccator maintaining $75 \pm 5$ per cent relative humidity by saturated sodium chloride solution for two weeks regulating the moisture level constantly. Percentage of damage by \textit{S. oryzae} and \textit{S. cerealella} in all the samples of rice cultivars were ascertained following the methods described by Prakash (1982) and Nigam \textit{et al.} (1987) for \textit{S. oryzae} and Anuradha \textit{et al.} (1989) and Verma and Uttam (1990) for \textit{S. cerealella}, with slight modification to suit the prevailing laboratory conditions.

For \textit{S. oryzae}, 50 g of rice grains of each rice cultivar were taken from the desiccator and counted. The grains were kept in glass vials (100 x 40 mm) separately in three replication for each cultivar. Ten pairs of 2-3 days old \textit{S. oryzae} were introduced in each glass vial and the vials were then covered with double layered muslin cloth and fastened with rubber bands. These vials were kept at $28 \pm 1 \degree C$ temperature and $75 \pm 5$ per cent relative humidity in laboratory for a period of 45 days.

A similar experiment was laid out separately for \textit{S. cerealella}. In this study 1000 healthy grains of each rice cultivar were taken from desiccator and selected with the help of a hand lens and weighed. These grains were stored in glass vials (75 x 20 mm) separately. Ten pairs of \textit{S. cerealella} adults of uniform age were released into each glass vial, the mouth of which was closed with double layered muslin cloth. The vials with each cultivar (in triplicate) were arranged in completely randomised design and kept at laboratory temperature $28 \pm 1 \degree C$ and $75 \pm 5$ per cent relative humidity for a period of 45 days.
Plate III

Mainagiri  Ranjit
Basmati     Balam
Joha        Pankaj

Plate III. Popular rice cultivars grown in Barpeta District
Plate IV. Popular rice cultivars grown in Barpeta District

Kolajoha
Bora
Katisali
Hasakumra
Diga (Bao)
Aijong
Both the experiments were laid out in complete Randomized Design with 12 treatments and three replications. At the end of each experiment the percentage of damaged grains of each glass vial was ascertained by physical examination as stated elsewhere.

The data of each experiment were subjected to analysis of variance.

3.7.2 Physical characters of rice cultivars

Physical grain characters of the 12 rice cultivars viz., length-breadth ratio, thickness of husk, pilosity (number of hairs on the grain surface) and length of the hairs were studied in all the 12 rice cultivars. All the physical grain characters studied were correlated with the per cent of damaged grains by the two species of insects.

3.7.2.1 Length - breadth (L / B) ratio

The length - breadth ratio of the grain was calculated by dividing the length (mm) of the grain by its breadth (mm). The average record of 20 grains in each cultivar was considered.

3.7.2.2 Thickness of grain husk

The thickness of grain husk (mm) was measured by using scale micrometer. Here average record of 20 grains in each cultivar was considered.

3.7.2.3 Pilosity (number of hairs) and length of hairs

The pilosity and length of hairs on the grain surface were measured under a compound microscope with the help of ocular and stage micrometers.
In using the ocular and stage micrometers the value of the ocular division was found out by calibrating the divisions of ocular scale with those of the stage micrometer scale. Each reading taken with the ocular micrometer was then multiplied with this value to find out the actual measurement in milimeter. The average record of 20 grains in each rice cultivar was recorded.

3.7.3 Chemical constituents of grains

3.7.3.1 Preparation of sample for chemical analysis

Samples of paddy were drawn just before their storage. The paddy samples of 12 different rice cultivars thus drawn were crushed to make powder in a mini mill so as to pass through 40 mesh sieve. The powdered samples were then oven dried for overnight at a temperature of 70°C. After drying, the samples were kept in desiccators. Estimation of each chemical constituent was carried out from three composite samples.

3.7.3.2 Protein content

Protein content (crude protein) was estimated following the method of Chopra and Konwar (1976).

3.7.3.3 Total soluble sugar content

Total soluble sugar content was estimated by A.A.C.C. method (1972).

3.7.3.4 Silica content

Silica content was estimated by the method of Anon. (1976).

Data on all the chemical constituents studied were correlated with the percentage of damaged grains by the two insect species.
3.8 **Life cycle of *Sitophilus oryzae* Linn. and *Sitotroga cerealella* Oliv.**

The detailed investigations on the life cycle of *S. oryzae* and *S. cerealella* in rice (cv. 'Hasakumra') were carried out at the Department of Zoology, Gauhati University under culture conditions during 1999-2000.

### 3.8.1 *Sitophilus oryzae* Linn.

The stock culture of *S. oryzae* was prepared by collecting large number of adults from farmers' receptacles of Barpeta district. Two hundred adult weevils were allowed to breed on 250 g freshly harvested polished rice (cv. 'Hasakumra') and kept in plain glass jars (16 x 10 x 8 cm) with their mouth tied with doubled layer muslin cloth. The average moisture content of the rice grains used for initiating the culture was adjusted to 14.5±0.1 per cent as recommended by Ryoo and Cho (1988). The culture of *S. oryzae* was maintained at laboratory temperature and relative humidity (24-32°C, 70-84 per cent RH) (Plate II). The laboratory temperature varied from a minimum average of 24°C to a maximum average of 32°C during the culture period. The relative humidity varied between a minimum average of 70% RH to a maximum average of 84% RH.

Freshly harvested polished rice (cv. 'Hasakumra') was used to culture the different life stages of *S. oryzae*. The collected rice grains from farmers' receptacles were first subjected to a temperature of 55°C±1°C for 4 hours in an oven to free them from other insect pests.

Eggs were obtained by using slightly modified methods of Richards (1947) and Howe (1952) which allowed to use grains containing one insect only, so that one weevil can complete development in a single grain.
Twenty mated females were isolated from stock culture jars (16 cm X 10 cm) to a glass tube (5 cm x 1.5 cm) with a single grain and allowed to lay eggs over 20 successive days at laboratory conditions (temperature 24°C to 32°C and relative humidity 70 to 84%). The twenty grains obtained daily were shared between five glass tubes (5 cm x 1.5 cm). The moisture content of the infected grains was adjusted to 14.5±0.1 per cent by maintaining the glass tubes in desiccators in which the normal humidity at 70% RH was controlled through saturated NaCl solution (Ryoo & Cho, 1988). The eggs could be easily detected in the grains, under a stereo binocular microscope, with fair ease by locating the holes plugged with gelatinous material as was also adopted earlier by Pant and Gupta (1959). The grains with gelatinous material containing the egg inside were randomly sampled (daily ten grains, in order to obtain large number of eggs) and stained (Frankenfeld, 1948) and those grains were carefully broken under a stereo binocular microscope with the help of needle and forceps. The egg thus obtained were used for observations in the present investigations. The grains with gelatinous material containing the egg inside were used for further studies of life cycle.

The detection of newly hatched larvae could be easily done from the appearance of conspicuous chalky white spots at their points of entrance into the grains. Thus for obtaining large numbers of newly hatched larvae, the rice grains developing chalky white spots on different dates were separated and carefully broken under a stereo binocular microscope with the help of a needle and forceps for further observations. The mean incubation period was determined by recording the date on which more than 50 per cent of the grains showed the next stage and the range of concerning later stage was fixed from that day on which the concerned stage was first seen upto the day on which only the subsequent stages were noticed.
To observe the number of instars and each duration, about 200 healthy sterilized grains were selected from the desiccators and 200 newly hatched (0-24 hours old) larvae were transferred with the help of a camel hair brush to host grains. Since the larvae feed within the grains, a single larva was placed inside each grain by making a small hole in the grain with a fine needle (Teotia and Singh, 1968). The newly hatched larvae entered the grains through the holes. The grains with introduced larvae were taken in specimen tube (7.5 x 3.5 cm) closed the mouth with muslin cloth and kept for further observations. The moisture content of the grain was adjusted to 14.5±0.1% (Ryoo and Cho, 1988).

From the next day onwards, daily 5 grains were taken and carefully dissected under a stereo binocular microscope with the help of a needle and forceps and the stage of larvae inside each grain was determined. Additional grains in lieu of non-infested grain or grains containing dead stage were also taken whenever necessary so as to have five observations per day. The development of conspicuous chalky white spots in the grains at the entrance point of introduced larva (and the spots) increased further as linear streaks with the advancement of the age of the larvae. The larva was carefully drawn out from the grain and studied under microscope. Presence of moulted skin and head capsule inside the grain were evidence as to the moulting of the larva and measurement of head capsule width of the larva were used to determine the larval instar. The mean duration of each instar was fixed as the day on which more than 50 per cent of the grains showed the next stage and the range was fixed from that day on which the concerned stage was first seen up to the day on which only the subsequent stages were noticed.
The development of hardened shell around the larva marked the end of larval stage and beginning of the pre-pupal stage. The dissections were continued till the day on which all the five grains showed the presence of pupae. Pupal period was easily determined by making regular observations every 6 hours.

Freshly emerged adults were transferred daily in separate glass tubes containing fresh healthy grains for studying the pre-mating and mating period, pre-oviposition and oviposition period, fecundity and adult longevity.

The experiment was carried out for three generations under laboratory conditions (temperature 24°C to 32°C, relative humidity 70% to 84%). The laboratory meteorological data are presented in Appendix I.

3.8.2 *Sitotroga cerealella* Oliv.

The stock culture of *S. cerealella* was obtained from the farmers' receptacles of Barpeta district. These moths were released on freshly harvested husked rice grains (cv. 'Hasakumra') and kept in glass jars (16 cm x 10 x 8 cm) for maintenance of culture.

Eggs were obtained by using suitably modified method of Sundararajan (1978) which allowed for more space for egg laying. Plastic jars (16 x 10 x 8 cm), were taken and provided with a small hole on the side for the purpose of introduction of moths. The lid possessed paired slits which were used for inserting the oviposition cards. Each oviposition card consisted of three strips of stiff black paper (6 cm x 2 cm) held together by clips. Of the three, in two of the strips diamond shaped portions of the paper were removed from the interior. These two strips were used to sandwich a plain paper without any such cuts in between,
which provided crevices all along the cut margins. A cotton swab dipped in 5 per cent honey solution was stuck to the inner side of the jar. Sometimes sugar solution was provided through the small hole on the jar used for moth introduction. Fifty mated females were isolated from stock culture, ten numbers each in a plastic jar with the freshly harvested dehusked rice grains of cultivar 'Hasakumra' and allowed to lay eggs at laboratory conditions. The eggs thus obtained were used for conducting the various studies observations in the present investigation.

Freshly harvested husked paddy grains (500g) were kept in an incubator with trays of water for two weeks in order to stabilize the moisture content of the grains. To determine the incubation period 50 newly laid eggs were carefully placed inside egg laying tubes (6 cm x 2 cm) along with adequate number of paddy grains collected from incubator. The open end of the egg laying tube was covered with muslin cloth held in position by rubber bands. The incubation period was noted by making regular observations at every 6 hours.

*S. cerealella* is an internal borer spending both larval and pupal stages inside the kernels. To determine the number and durations of different instars, first 200 healthy grains were selected from conditioned grains kept in incubator. The freshly emerged first instar larvae (0-8 hr. old) were carefully allowed to enter through a hole drilled using a fine needle at the germ region of the grain. Grains with introduced larvae were taken in glass petridishes (4 cm) in laboratory on the same day for further observations.
From the next day onwards, daily 5 grains were dissected under a stereo binocular microscope and the stage of larva inside each grain was determined. Additional grains in lieu of noninfested grain or grains containing dead stage were also taken whenever necessary so as to have five observations per day. The husk was removed in the first instance and then using a fine bent needle the larva was drawn out of the kernel for observation. Presence of moulted skin and head capsule inside the seed were the evidence of moulting of the larva and these were made use of to ascertain the larval instar. The mean duration of each instar was fixed as the day on which more than 50 per cent of the grains showed the next stage and the range was fixed from that day on which the concerned stage was first seen upto the day on which only the subsequent stages were noticed. The appearance of circular membraneous areas on the grain called "emergence windows" marked the end of larval stage and beginning of pupal stage. The dissections were continued till the day on which all the five grains showed the presence of pupae. Pupal period was recorded by making regular observations in every 6 hours.

Freshly emerged adults were transferred daily to separate (4 cm) petridishes containing fresh healthy grains for studying the pre-mating and mating, pre-oviposition and oviposition periods, fecundity and adult longevity.

This investigation was continued for three generations under laboratory temperature (24° to 32°C) and humidity (70-84%) conditions. The laboratory meteorological data are presented in Appendix I.
3.8.3 Morphometrics of *S. oryzae* and *S. cerealella*

The length and breadth of eggs, larvae, prepupae and pupae were measured with the help of ocular-stage micrometer and under 10X magnification of a compound microscope. The value of one ocular division was found out under the microscope by calibrating the divisions of ocular scale with those of stage micrometer scale. Each reading taken with ocular micrometer was then multiplied with the value of one ocular division to find out the actual measurement in mm. For studying the morphological characters and the morphometry of various stages of *S. oryzae* and *S. cerealella*, larvae and adults were killed by exposing them to carbon tetrachloride-soaked cotton swab in killing bottle. The widths of the egg, head capsules and various developmental stages were measured at the broadest part of the body.