3. MATERIAL AND METHODS

3.1. Materials

1. Culture of *Sesamia inferens* was collected from the farm of National Dairy Research Institute (NDRI), Karnal and maize fields of village Atterna near Sonepat, Haryana. The culture was multiplied and maintained in the Entomology Laboratory, Directorate of Maize Research (DMR), Pusa Campus, New Delhi.

2. Twenty lines of maize (Table 3a) were obtained from Winter Nursery Centre, Hyderabad.

### Table 3a. Twenty maize germplasm

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Pedigree</th>
<th>Code No.</th>
<th>Pedigree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E4-C</td>
<td>11</td>
<td>HKI PC4B</td>
</tr>
<tr>
<td>2</td>
<td>E5-O</td>
<td>12</td>
<td>HKI 1040-5</td>
</tr>
<tr>
<td>3</td>
<td>E9-B</td>
<td>13</td>
<td>HKI 1040-11-7</td>
</tr>
<tr>
<td>4</td>
<td>E30</td>
<td>14</td>
<td>Hyd05R/2-1</td>
</tr>
<tr>
<td>5</td>
<td>E37-A(O)</td>
<td>15</td>
<td>HKI C323</td>
</tr>
<tr>
<td>6</td>
<td>E57(0)</td>
<td>16</td>
<td>HKI 164-7-4 ER-3</td>
</tr>
<tr>
<td>7</td>
<td>WNZPBTLL6</td>
<td>17</td>
<td>PFSR-S3</td>
</tr>
<tr>
<td>8</td>
<td>E 60(FC)O</td>
<td>18</td>
<td>PFSR-R9</td>
</tr>
<tr>
<td>9</td>
<td>AEB(Y)C5 55-1</td>
<td>19</td>
<td>Basi Local</td>
</tr>
<tr>
<td>10</td>
<td>AEB(Y)C5 34-1</td>
<td>20</td>
<td>CM-202</td>
</tr>
</tbody>
</table>
3. The preliminary studies on ovipositional behaviour were done in versatile insect rearing cages (VIRC) (Kumar et al., 2012).

4. Studies on ovipositional preference and conventional screening by artificially infesting the plants using neonate larvae were conducted under controlled conditions in Glasshouse of Directorate of Maize Research, New Delhi.

5. Insect culture room was maintained at 27 ± 1 °C and 70 - 80 per cent relative humidity. Incubator (0°C-50°C), refrigerator, thermometer, blenders with 500 mL, 1L and 5L capacity, hot air oven (50°C to 250°C), hot plate, weighing balance, measuring cylinders 1, 10, 100 and 1000 mL capacity, round stainless steel bowl (23 cm dia. x 12 cm high), stainless steel spatula, markin cloth, insect proof ventilated almirahs, trays, dehumidifier, air cleaner, rubber bands, black paper, tissue paper, glass jars (15 cm x 10 cm and 15 cm x 20 cm), petri dishes (5 cm, 10 cm, 15 cm dia.), sand, scissors, camel hair brush etc. were the items used in the study.

3.2. Methods

3.2.1. Nucleus Culture of S. inferens

The nucleus culture of S. inferens was collected from NDRI, Karnal and village Atterna near Sonepat, Haryana and maintained in the Entomology Laboratory, DMR under the constant rearing environment at a temperature of 26 ± 2.0°C and relative humidity of 65 ± 5%. The field collected larvae were reared on fresh maize stalk and baby corn till pupation. After emergence, the male and female adults in equal numbers were released on potted maize plants (7-12 day old) kept in VIRC. These eggs were used in mass rearing of S. inferens. The larvae were reared on artificial diet (Siddiqui et al., 1977). Rearing of larvae on natural diet is not only cumbersome but
excessive handling predisposes the larvae to mechanical injury. Besides, fresh plant material may not be available all the time, hence the culture was reared and maintained on semi synthetic diet.

**Semi-synthetic diet:**

The base material of diet was green gram powder, wheat flour in agar medium. The diet was provided by antibacterial and antifungal preservative and fortified by vitamins. The list of ingredients is given below.

**Table 3b. Diet ingredients for *Sesamia inferens***

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Ingredients</th>
<th>Quantity (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Fraction A</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Green gram grain powder</td>
<td>70.0</td>
</tr>
<tr>
<td>2</td>
<td>Wheat flour</td>
<td>18.0</td>
</tr>
<tr>
<td>3</td>
<td>Maize leaf and whorl powder</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>Sucrose</td>
<td>2.0</td>
</tr>
<tr>
<td>5</td>
<td>Brewers yeast</td>
<td>5.0</td>
</tr>
<tr>
<td>6</td>
<td>Vitamin E</td>
<td>0.1</td>
</tr>
<tr>
<td>7</td>
<td>Ascorbic acid</td>
<td>1.7</td>
</tr>
<tr>
<td>8</td>
<td>Methyl-p-hydroxy benzoate</td>
<td>0.8</td>
</tr>
<tr>
<td>9</td>
<td>Sorbic acid</td>
<td>0.4</td>
</tr>
<tr>
<td>10</td>
<td>Formaldehyde 40%</td>
<td>1.0</td>
</tr>
<tr>
<td>11</td>
<td>Water</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td><strong>Fraction B</strong></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Agar powder</td>
<td>6.0</td>
</tr>
<tr>
<td>12</td>
<td>Water</td>
<td>130</td>
</tr>
</tbody>
</table>
Brand Name

Agar – M/S Cellulose Product of India Ltd., Ahmedabad.

British Drug House/ Glaxo Laboratories, Mumbai.

Yeast powder - Local pack

Ascorbic acid – M/S Sarabhai M. Chemicals, Baroda.

Sorbic acid – M/S E Merck, Darmstadt, Germany.

Methyl-p-hydroxy benzoate – M/S E Merck, Dannstadt, Germany.

Vitamin E (Viteolin capsule) – M/S Allenbury’s Pharmaceutical Division and Division of Glaxo Laboratories Ltd., Mumbai.

Formalin – M/S Indian Drugs and Pharmaceutical Ltd., Hyderabad.

Wheat - Local purchase

The jars, paper and marking cloth used for rearing the larvae were sterilized in oven at 120°C for an hour. Brushes used for handling neonates were sterilized everyday by dipping them in 70% alcohol. All the ingredients of Fraction - A were weighed and transferred into stainless steel jar of a blender and blended in 260 mL of distilled water till the time it became homogeneous slurry. The weighed quantity of agar of Fraction - B was then taken in a beaker and heated to boiling for 5-6 minutes. The hot agar solution was added to the contents of the jar and blended for 2-3 minutes. The diet mixture was poured into sterilized glass jars (15 cm x 10 cm) to a depth of 3-4 cm. The jars were covered with sterilized paper and kept at room temperature for about 12 to 24 hr during which the diet gets solidified.
Larval rearing:

The rearing of pink borer was carried out in the laboratory at 26 ± 2 °C and 70 - 80 % relative humidity. 40 - 50 neonates were released in the artificial diet in jar (Plate 1). The mouth of each jar was covered with sterilized markin cloth and the wall with black paper from all the sides except the lower end of the jar containing diet. The black paper was secured in place using rubber band. The light enters the jar only through uncovered area. The covering of jars was intended to direct the larvae towards light and hence on to the diet so that they get established on it and do not dissipate their energy in search of food. The diet jars were monitored for any microbial growth at alternate days. The larvae were provided with the fresh diet as and when required.

Precautions were taken to sterilize all the materials very meticulously, which come in contact with larvae such as jars, paper, markin, cloth etc. As a matter of practice, the material is sterilized in the afternoon and the oven is switched off before leaving the laboratory in the evening. The material is removed from the oven the next day. Care was taken not to remove the hot jars from the oven lest they crack. Brush used for handling egg mass or larvae is often major source of infection. They were, therefore, sterilized by dipping them in 70% alcohol before using them. The larval development was completed in the same jars in which neonates were released.

Pupal handling

When most of the larvae turned into pupae, they were collected and segregated for their sex based on their size. The pupal cages were prepared by placing 2-3 inch thick layer of sand which was moistened by spraying sufficient quantity of water over it. The sand surface was covered by a circular butter paper. The top of the jar was
Rearing of *Sesamia inferens*

**PLATE I**

A- Oviposition cage for adult rearing  
B- Eggs inside leaf sheath  
C- Eggs hatching  
D- Release of neonates in artificial diet  
E- Larval rearing on artificial diet  
F- Larval rearing on maize stem and baby corn  
G- Pupal jars  
H- Plants as substrate for oviposition of adults
covered with markin cloth. The development of male pupae are faster than that of females, therefore, the male pupae were kept at low temperature (15°C) for a day or two so as to synchronize the moth emergence with female pupae.

**Adult rearing and Egg harvest**: One pair of freshly emerged moth was released over 7-12 day old potted maize plants (2 plants in each pot) caged in VIRC. These cages were kept in culture room at 26± 2°C. The ovipositoon cages were kept moist to achieve 70-90% humidity by keeping water plate under the cage. The egg laying was complete in 4-6 days. The plants were removed and the portions of leaf sheaths containing eggs were cut and kept at 26± 2°C for incubation.

3.2.2. **Screening of germplasm**

**OBJECTIVE 1: Determination of susceptible age of the plant for oviposition**

The most susceptible plant-age study was conducted using two germplasm HQPM1 (the resistant) and Basi Local (the susceptible) in multi-choice and no-choice situation replicated five and three times respectively. Six ages viz., 4, 8, 12, 16, 20 and 24-day old plants were used for this study.

**Multi-choice test** - Profile of six ages of each germplasm were raised by staggered sowing in plastic pots at four-day interval up to 24 days in glass house during Spring, 2012 so as to obtain the plants of 4, 8, 12, 16, 20 and 24 day-old plants at a time for executing the experiment. Six pots of each age were arranged in a circular manner in VIRC to give each plant equal chance of being selected for oviposition by the moths released in centre. One pair of laboratory reared, 1-day-old *S. inferens*moths was released in the oviposition cage in the morning (0900). This set of test was repeated 5 times.
**No-choice test** - Four plants of same age were arranged similarly in a circular manner in VIRC to give each plant equal chance of being selected for oviposition by the moths released in centre. This set of experiment was replicated 3 times for each age. One pair of laboratory reared, 1-day-old *S. inferens* moths was released in the oviposition cage in the morning (0900).

**Data recording**

At the end of 5th day, each plant in cage was cut from the base, properly labeled and brought to laboratory to count the number of egg masses per leaf sheath and number of eggs per egg mass laid in five days. The average number of eggs per plant in each age was then calculated.

**OBJECTIVE 2: Determination of plant to insect ratio for oviposition studies**

**Experiment 1: To determine the number of plants oviposited by one mated female**

Ten VIRC each having ten pots with one 12-day old plant of HQPM1 in each pot were taken. One freshly-emerged adult pair of *S. inferens* was released in each oviposition cage. The plants were watered as and when required to maintain them in good condition. After five days, the plants were taken out and observed for egg masses. The number of egg masses in each plant was recorded. The eggs in each egg mass were also counted and recorded.

**Experiment 2. Distribution pattern of eggs among and within the plant**

A susceptible germplasm of maize, Basi Local and resistant germplasm, HQPM1 were used for this study. To study the distribution pattern of eggs on variable number of plants, the experiment was conducted in four sets; each set having 1, 2, 3 and 4
number of 12-day old plants kept in VIRC. In each cage one-day old pair of *S. inferens* adults was released. This was replicated three times. On fifth day, the pots were taken out of the cage and number of infested plants in each cage were counted and recorded. The number of egg masses laid on each plant were also counted and recorded. The leaf sheaths were removed to count the number of eggs in each egg mass.

**Experiment 3: Age specific fecundity of *S. inferens***

HQPM1 was used to study the age specific fecundity. Staggard sowing of 20 plants was done at alternate days for 10 days. Two 12-day old plants were kept in insect rearing cage and freshly emerged *Sesamia* moth were released on these plants. The plants were replaced with two fresh 12-day old plants everyday for the same pair of adults. The number of eggs and egg masses laid by *S. inferens* in the removed plants were recorded. The experiment was replicated seven times. The removal of old plants and replacement with fresh plants were done daily in the morning hours and continued till the female survived.

**Statistical analysis**

The data obtained were averaged for each treatment and subjected to one-way analysis of variance (ANOVA) using SPSS16 software using least square design.

**OBJECTIVE 3: Ovipositional preference of *Sesamia inferens* to different germplasm**

**Multi-choice test**

The ovipositional preference of *S. inferens* was studied on twenty selected germplasm. For each germplasm six seeds were sown per pot of size 30 x 20 x 10 cm in glass house. A set of three such pots for each germplasm was prepared. Four plants
Ovipositional preference of *S. inferens*

Multi-choice test

Plate II
were maintained in each pot. Thus three pots each containing 4 plants of each germplasms constituted three replications. The following observations were recorded before releasing the adults:

I. Plant Height

II. Stem diameter near first leaf sheath

One set of twenty pots of twenty germplasm was kept under screening cage (mosquito net) of size 180 cm x 180 cm x 120 cm (Plate 2). The set of twenty pots made one replication. Three such screening cages were set up in the glass house. When the plants became 12-day old, twenty pairs of moths were released inside each screening cage. After 5 days, all the plants were cut, germplasm wise labeled and brought to laboratory. The following observations were recorded.

i) No. of egg masses per plant

ii) No. of eggs per egg mass

iii) No. of eggs per leaf sheath

iv) No. of plants oviposited

No-choice test

The same set of twenty genotypes was planted in plastic pots. Six seeds were sown in six pots and four plants were maintained. Four pots each having one 12-day old plant of same germplasm were kept in VIRC (Plate 3). One pair of laboratory reared, 1-day old *S. inferens* moths was released in each oviposition cage in the morning (0900 h). This set of experiment was replicated thrice. After five days, the plants were cut, labeled and brought in laboratory and following observations were recorded.

i) No. of egg masses per plant

ii) No. of eggs per egg mass
Ovipositional preference of *S. inferens*

No-choice test inside glass house

PLATE III
iii) No. of plants oviposited

**Statistical analysis:**

The design of the experimental set up was completely randomized design (CRD). ANOVA was done to categorize germplasm according to level of susceptibility based on the above stated parameters using SPSS 16. Trends of various parameters were analysed to determine the relative variation in susceptibility among these germplasm. The multiple correlations between the parameters were determined to study the relationship between the parameters.

**OBJECTIVE 4: Determination of susceptibility to larvae in maize germplasm**

**Treatments: 20 (20 germplasm)**

**Replication: 3 (Three pots, 5 plants in each pot)**

For each germplasm, 45 plants were sown in 9 pots with five plants per pot of 30*20*10 cm. Thus 15 plants of one germplasm grown in 3 pots constituted one treatment (Plate 4). Twelve days after the germination, five neonates were released on each plant near the first leaf sheath (Plate 5). On 7th day after infestation, fifteen plants from three pots were dissected to recover the larvae. The number of recovered larvae and their weight was recorded. Again on 15th day after infestation, second disective sampling of 15 plants from three pots was done to recover the developing larvae and their number and weight was recorded. In the remaining set of 15 plants, the visual grading of leaf injury was done in the 1-9 scale (1-healthy plant, 9-dead heart) on 21st day after infestation. After taking observations for LIR, the plants were dissected in the laboratory to recover the developing larvae. The recovered larvae were collected and weighed individually. These larvae were then reared on natural food common for
Maize germplasm inside glass house for antibiosis studies

PLATE IV
Infestation of maize germplasm with neonates
Inset (Neonates used for infestation)

PLATE V
all to get the pupae. Observations on larval period, pupal period percent larval survival and pupal weight were recorded.

The standard scale used for leaf injury rating was as follows:

**Table 3c. Leaf Injury Rating and level of susceptibility**

<table>
<thead>
<tr>
<th>Visual Symptoms</th>
<th>LIR</th>
<th>Level of susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparently healthy plant</td>
<td>1</td>
<td>Least Susceptible</td>
</tr>
<tr>
<td>Plants showing slightest damage of leaf or few pin holes on 1-2 leaves</td>
<td>2</td>
<td>Less Susceptible</td>
</tr>
<tr>
<td>Plants showing more pin holes or shot holes on 3-4 leaves</td>
<td>3</td>
<td>Less Susceptible</td>
</tr>
<tr>
<td>Plants showing injury (pin holes, shot holes and slits) in 1/3 of total number of leaves and mid-rib tunneling on 1-2 leaves</td>
<td>4</td>
<td>Moderately Susceptible</td>
</tr>
<tr>
<td>Plants showing 50% leaf damage (pin holes, shot holes, slits, streaks) and mid-rib damage</td>
<td>5</td>
<td>Moderately Susceptible</td>
</tr>
<tr>
<td>Plants showing leaf injury in 2/3 of the total number of leaves</td>
<td>6</td>
<td>Moderately Susceptible</td>
</tr>
<tr>
<td>Plants with every type of leaf injury and almost all leaves damaged</td>
<td>7</td>
<td>Highly Susceptible</td>
</tr>
<tr>
<td>Entire plant showing maximum leaf injury and likely to form dead hearts</td>
<td>8</td>
<td>Highly Susceptible</td>
</tr>
<tr>
<td>Dead hearts (total damage to plant)</td>
<td>9</td>
<td>Highly Susceptible</td>
</tr>
</tbody>
</table>

**Statistical analysis:**

The design selected for the conducting the experiment was completely randomized design (CRD). ANOVA was done to categorize germplasm according to level of susceptibility by studying the biological parameters of *S. inferens* and LIR using
SPSS16. Both individual and multiple correlations between the parameters were determined to study the relationship between the parameters.

**OBJECTIVE 5: Correlation between ovipositional preference and susceptibility to larvae**

The correlation between antixenosis in terms of ovipositional preference parameters and antibiosis in terms of germplasm susceptibility level to neonates was determined to know the extent of relationship between them. The susceptibility index was developed for antixenosis (SI-X) and antibiosis (SI-B). The following parameters were selected for calculating the above mentioned susceptibility indices. The total (cumulative) susceptibility index (SIT) was calculated by combining both the SI for the antixenosis (SI-X) and antibiosis (SI-B) as given below:

**Table 3d: Parameters selected for determining SIT**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Antixenosis parameters</th>
<th>Antibiosis parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Multi-choice</td>
<td>No-Choice</td>
</tr>
<tr>
<td>1</td>
<td>Total number of eggs</td>
<td>Total number of eggs</td>
</tr>
<tr>
<td>2</td>
<td>No. of plants oviposited</td>
<td>No. of plants oviposited</td>
</tr>
<tr>
<td>3</td>
<td>No. of egg masses</td>
<td>Number of larvae recovered per plant</td>
</tr>
<tr>
<td>4</td>
<td>No. of leaf sheaths</td>
<td>Larval weight (mg)</td>
</tr>
<tr>
<td></td>
<td>oviposited</td>
<td>Larval period</td>
</tr>
</tbody>
</table>

*Formulation of Indices of susceptibility*

The original data for each parameter of antixenosis and antibiosis, except development period, was transformed to their relative value on 0-1 scale using the formula \((X - \text{Xmin})/(X\text{max} - \text{Xmin})\), where \(X\) is the data, \(\text{Xmin}\) and \(\text{Xmax}\) are the lowest
and the highest value respectively of the range of data. In case of development period, the data were first inverted by using formula $X_{\text{max}} - X$ to have positive relation with susceptibility. This data was then transformed on 0 to 1 scale by using the formula used for other parameters. All the ten parameters were then summed up to arrive at a total susceptibility index (SIT).

**Susceptibility index for antixenosis (SI-X)** was computed as the sum of susceptibility index based on multi-choice (SI-MC) and no-choice (SI-NC).

$$\text{SI-X} = \text{SI-MC} + \text{SI-NC}$$

Where

$$\text{SI-MC}= \frac{\text{Total number of eggs laid/germplasm} + \text{Number of plants ovipsoited} + \text{Total number of egg masses/germplasm} + \text{Number of leaf sheaths oviposited}}{\text{germplasm}}$$

and

$$\text{SI-NC}= \frac{\text{Total number of eggs laid/germplasm} + \text{Number of plants ovipsoited}}{\text{germplasm}}$$

**Susceptibility index for antibiosis (SI-B)**

$$\text{SI-B} = \text{LIR} + \text{Number of larvae recovered per plant} + \text{Average weight of larva (mg)} + \text{Average larval period (days)}$$

**Total susceptibility index of germplasm (SIT)** was computed as sum of susceptibility index of antixenosis (SI-X) and susceptibility index of antibiosis (SI-B).

$$\text{SIT} = \text{SI-X} + \text{SI-B}$$

The extent of relationship between them was determined using the statistical package SPSS16. The test germplasm were categorized based on the susceptibility indices.