Chapter-3
Research Methodology

3.1 Research Methodology for Survey and Sampling
3.2 Collection of Natural Enemies from Different Outfields
3.3 Rearing of Sampled Insects
3.4 Establishment of Research Laboratory
3.5 Laboratory Bioassays
3.6 Methods of Stock Collection
3.7 Taxonomic Research Study
3.8 Laboratory Processes
3.9 Research Methodology for Cultivation of Chilli Plants
3.10 Biological Controlled Experiments of Thrips Under Net House Conditions
Chapter-3
Research Methodology

Biological Control - is a Term & Approach

It is a rapidly growing approach which brings together ecologists, entomologists, weed scientists, plant pathologists and microbiologists. Biological control is a manipulation through nature and natural agents.

These are beneficial organisms that reduce pests and diseases. They are called Farmers’ Friends. They can be conserved by taking care with farming practices.

Keeping in view the several existing hypothesis and research efforts of researchers published in various journals, experiments have been made on the host plant Chilli, Capsicum annuum. These experiments have been carried out on Chilli thrips, S. dorsalis, and their host plant chili, Capsicum annuum. These experiments were carried out with reference to biological control with their natural enemies; predators and parasitoids.

Various aspects and legislative approaches have been considered to accomplish our research work. Points in favour of ecology and human health have been kept in mind in the following manner:

- Checked pest; Thrips population and level of fluctuation on Chilli crop by the exploring survey of Chilli outfields.
- Evaluated the natural occurrence of natural enemies from selected outfields.
- Maintained the research aspects of our objectives around the Tritrophic Interaction between Host plant; Chilli, Pest - Thrips and Natural Enemies: Predators and Parasitoids.
- Used Random Sampling and Tapping Methods to check the fluctuation and collection of thrips and their natural enemies; Predators and Parasitoids.
- Maintained Experiemntal Net house near research laboratory to controlled the infestation caused by thrips by augmentative biological control release.
Performed Taxonomical and Biological studies of thrips and natural enemies.

Explored and evaluated our methodological aspects on Chilli crop with the help of selected predators and parasitoids.

### 3.1 Research Methodology for Survey and Sampling

To accomplish our research objectives regarding checking of the pest population on Chilli crop and for the presence of their natural enemies, we selected those localities, where farmers used less pesticides. In this connection, the five selected sites in District Aligarh of Western Uttar Pradesh namely; Talib Nagar, Jalali, Tappal, Sumera, Kayamganj were taken.

The conditions of the decided outfields were in favour of sampling the chilli plants. Therefore, total five outfields covered our whole research. The surveys were made during the morning hours and in the favourable conditions of chilli crop. Survey was started in the month of August, 2014 and lasted on August, 2015. During survey, the growth in the population of chilli thrips on chilli crops was observed from seedling stage till harvesting stage. Survey and observation interval was calculated “fortnightly”. In this manner, sufficient time was maintained from one time growth to another time growth.

For increasing demand in domestic and foreign markets, it deserves our serious consideration for higher production of agricultural crops. They form a part of daily diet in almost all households. These beneficial crops are prone to heavy infestations by thrips pests. In this regard we are concerned with the sampling of thrips. From the view of sampling we saw the population fluctuation of pests and its natural enemies.

**Population fluctuation of Thrips Pests**

It is important to check the presence of Thrips pests in the farmers’ fields during our research. For this sampling method was applied at regular time intervals. In the regulatory survey, we chose some chilli plants for measuring the intensity of thrips population.

**By Random Sampling Method** : It is a method of sampling in which each individual plant is chosen randomly and entirely by chance. So, each individual has the same probability of being chosen at any stage.
By using this method, the sampling has been done against the presence of targeted insects. Sampling plants were chosen randomly as, four in every direction, North, South, East, and West and fifth in Central Area of the selected chilli outfield. In this regard, we were also concerned with the sampling of natural enemies. So, in the same manner, we sampled the natural enemies; predators and parasitoids of Thrips. From farmers' fields we selected five plants from one outfield. In order to complete the process, an initial experimental unit was established.

**By Tapping Method – For Thrips:** In order to get better and more remarkable results we applied tapping method. By this method, samples of insects were collected. We gently tapped the plant leaves, twigs and flowers on white sheet of paper. In this manner, the thrips concentrated on the leaves were dislodged and could be seen on the white paper.

Seasonal Population Fluctuations of *Scirtothrips dorsalis*: A survey was made to check the population fluctuations of *S. dorsalis* on chilli crop at changing environment. For this purpose a regular survey for collection of thrips was completed during second half of August 2014 to the first half of August 2015. Collected data was tabulated according to collection sites. Simple tabular analysis was made to work out seasonal population fluctuation of thrips species.

**By Sticky Card Method:** Thrips were collected from their host plant at the time of infestation. The Thrips adults were picked up with the aid of camel hair brush. It was transferred to glass vial containing 70% ethanol and brought to the research laboratory in D.S. College, Aligarh.
3.2 Collection of Natural Enemies from Different Outfields

We collected a few natural enemies of thrips on the basis of our observations. Adults were directly handpicked from the chilli plants with the help of forceps.

Besides the collection of handpicked natural enemies, we maintained stock of insect fauna from other sources also with a view to finding out the method of biological control. A few species of parasitoids were collected for the purpose of exploration.

3.3 Rearing of Sampled Insects

The evaluation through the process of rearing and mass production of *S. dorsalis* was performed continuously under research laboratory of Zoology Department, D.S. College, Aligarh. In this manner, some numbers of thrips were also collected from different surveying sites of District Aligarh to maintaining our stock.

In the same manner the adults of selected predators and parasitoids were collected. On the other hand, a few species of selected predators and parasitoids were also collected during the rearing period of thrips under laboratory experiments when they were emerging from the infected eggs and larvae of the thrips. We also obtained some live species of natural enemies of thrips from the Department of Zoology, Aligarh Muslim University, Aligarh, Indian Agricultural Research Institute, and New Delhi and also from the National Bureau of Agriculturally important insects, Bangalore. In this manner, a stock of thrips (included all life stages) and their natural enemies were maintained for our experiments of biological control and its management against thrips population.

3.4 Establishment of Research Laboratory

The establishment of laboratory was a primary step to carry on our research work in the field of entomology with a view to discovering biological approaches with experiments on the host-plant pest i.e. thrips by their natural predators and parasitoids. The research laboratory was well equipped with appropriate scientific tools necessary for our purpose. The evaluated work
was carried inside our research laboratory under suitable conditions, whichever plants were collected from the outfields at regular intervals and time-to-time transferred in research laboratory. Under laboratory conditions, we processed and assessed the biology, the preservation, and taxonomic identification of thrips species from the genus and their natural enemies.

**Taxonomical Identification**

After completed the gradual dehydration, the thrips species were identified and their morphological traits were compared using taxonomic characteristics. These specimen characteristics were identified with the help of dissecting microscope at a minimum of 10x magnification.

### 3.5 Laboratory Bioassays

The use of laboratory bioassays was also made for the mass production, culturing and rearing of selected samples. Some used bioassays were as followed:

1. **Petri Dishes**

   From the selected sites the infected or parasitized eggs and larvae of thrips were collected and put in petri dishes with the help of camel hair brush. After some time, these petri dishes were transferred in BOD incubator for emerging of adult thrips.

2. **Glass Vials**

   Glass vials with alcohol were used to preserve the different life stages; eggs, larvae, pupae and adults of thrips. It was also used to collect the life stages of selected natural enemies. These thrips and natural enemies were collected from the different selected sites of District Aligarh of Western Uttar Pradesh.
(3) **Binocular Digital Microscope & Camera Lucida**

For the study of biology and taxonomy of selected thrips specimens and their natural enemies we used the Binocular digital Microscope. We also applied the camera Lucida to draw the scratch diagrams of the insect specimens.

![Figure 3.6: Binocular Digital Microscope & Camera Lucida](image)

(4) **Rearing Cages & Plexi Glass Containers**

These were made up of wooden cages. These wooden cages used for proper ventilation to thrips. Each cage opened by a sliding lid. A mesh in each cage was also fixed on lid to prevent the other insects.

Ventilated plexi glass containers with lids were purchased for rearing of thrips. Culture of thrips was also preserved in the containers for further scientific studies.

![Figure 3.7: Rearing Cage](image)

(5) **BOD Incubator (Biological Oxygen Demand)**

It is the method in which the amount of dissolve oxygen is needed by aerobic biological organisms against break down of organic material which present in a given sample at appropriate temperature over a specific period of time. BOD Incubator was used for rearing the selected specimens who were collected from selected outfields.

![Figure 3.8: BOD Incubator](image)
(6) **Insect collecting boxes**

These were used for the preservation and to maintain our record of selected sample specimens which were collected from the different outfields.

### 3.6 Methods of Stock Collection

(1) **Pinning of Predator Samples**

This method was used for preserving and handling insects for the taxonomical studies. Pinned insects were stored quite safely. For this, various sizes of entomological pins were purchased and used.

- Regarding this 2/3 portion of pin was used below the pinned insect and 1/3 above.
- In Hymenopterous insect predators were pinned through the centre of mesothorax.
- Some small collected insects were pinned by micropins; small sized pins.

(2) **Spreading & Positioning by spreading board**

The spreading board or setting board was used for some collected predatory insects. Predatory mites and minute pirate bugs were properly spread on the board under our laboratory conditions. In this process, the antennal was direct and frontal. Abdominal appendages and their ovipositor were directly backward. Wings were spread properly. Hind edge of forewing was at right angle to the body and hind wing appropriately matched with forewing. After this, the wing setting was done by pinning the paper stripes on the spreading board.

(3) **Carding Method**

Some collected small insects were placed on a white rectangular card; 5x8 mm or 5x12 mm. below this, card data labelled card was also placed in which we contained some information about the collection of the samples during the research survey. In this manner, information about identification of such insects, name of their host plant, date of collection, name of the collector
etc was properly placed. Some small triangular cards were also used for pinning the small insects.

![Figure 3.10: Carding with Triangular and Rectangular Cards](image)

### 3.7 Taxonomic Research Study

#### (1) Tools and Techniques

The evaluation and manipulation of specimens were arranged with the help of fine micropins, mounted with sealing wax on matchsticks. A pair of straight and with apex bent pins was used for taxonomic slide preparation. To accomplish our sample specimen’s simple lifting tool was used and movement was successfully done. It was made of a small loop of fine wire. Alcohols of the dipped specimens were changed in laboratory dishes by using a fine glass pipette.

![Figure 3.11: Tools used for evaluation and manipulation of taxonomic study](image)
(2) Process for Slide Preparation

For the taxonomical point of view, our objective was to prepare specimens on the slides with their natural shapes and colour. They were retained in a condition as close as possible to the natural position and living state. On the other hand, the body was clear and the surface details were visible.

Figure 3.12: Prepared Entomological slides for taxonomical study

For Routine Identification: The Routine Identification procedure was rapid and thus relatively inexpensive. The slides were prepared in such media solutions which could keep them intact for years, even though these slides were not permanent. It is a method which may be recommended for all routine identification work. In the present research work, this method was particularly used against small larvae and pupae and for small adults which were pale in colour. The Mounting and preserving steps were taken in the following manner:

1. Removal of the selected specimens from the collected fluid into 70% ethanol.
2. Specimens were reasonably flexible. So, they were attempted to open wings and straighten their antennae with the help of micropins.
3. After that we placed a drop of mountant solution over the specimen thrips and then they were covered with a cover slip of 13mm circle. Specimen thrips were placed in such a way that ventral slide was uppermost.

4. When the processed mountant became dry, we labelled them in appropriate manner.

Thrips specimens were mounted on microscopic slides by following method.

From the preservative solution thrips were taken and placed in 10% solution of cold sodium hydroxide. The intersegmental ventral abdominal integument was punctured in order to aid the alkali solution quickly reach the body contents and to make the procedure more efficient and faster. Small Terebrantion insects remained there for 5-15 minutes. This depended on the sample colour of their integument.

After the removal of specimens from that caustic solution, they were placed in a solution of acetic alcohol (50% ethanol, 4 parts glacial acetic acid, 1 part) and there they were allowed to remain for at least 12 hours.

In this manner, specimens were transferred to 70% alcohol in order to remove the acetic acid contents from the specimens. The wings, legs and antennae were arranged in a systematic manner. Then, the processed specimens were placed in a “syracuse” watch glass in 95% alcohol and weighted down with a piece of cover glass. In this manner, specimens were allowed to harden at least over-night.

We arranged three small round bottomed porcelain dishes which were about 2.5 inches in diameters for the purpose of keeping the processed specimens intact.

- The first bottomed porcelain dish, contained 95% ethanol.
- The second dish contained half 95% ethanol and half amount of xylene.
- In the third porcelain dish we contained pure xylene.

These specimens were left in dish first for at least 10 minutes and then transferred to second dish for 10 minutes.
In the process of mounting use the fine camel hair brush was used to avoid damage on the specimens when moved from one dish to another. After one minute, they were placed in the third dish and were allowed to remain for 1-1.5 minutes. It depended on the specimen and our observation.

In that series of mounting, a drop of thick balsam was placed on a slide and specimens were transferred to it. In order to hold the wings and legs in outspread position use of thick balsam was made. Efforts for it were made for proper arrangement of wings, legs, and antennae. This arrangement was necessary because we needed for extensive taxonomical study of thrips specimens during the research.

**Suggestion:** The cover slip which would be used should be small because large cover slips crush specimens and also need more mountant solution.

### 3.8 Laboratory Processes

(1) **Maceration: The Process**

The objective of this process in our research was to remove the body contents of sampled specimens. Maceration process was done by soaked specimens in a weak NaOH solution for a appropriate period. The preference of NaOH solution was in favour of less damage to the body surface compared to KOH solution. The treatment of Maceration process was maintained for the period which was necessary for our research programme. The process was charted according to the suggestions and advice of the entomological experts.

(2) **Dehydration: A Process**

The main purpose of this process is to remove water from specimens. The specimens clearing were improved by massaging each specimen gently with the help of the back of the bent needle. At this point we followed the following steps:

1. Replaced the 60% alcohol with 70% alcohol. It was left for 1 hour.
2. Unmacerated specimens were punctured for speeding up the entry of alcohols. It was the way to spread the insect legs, antennae and wings.
3. Then replaced with 80% alcohol. It was left out for 20 minutes.
4. Replaced with 90% alcohol. It was left for 10 minutes.
5. Replaced and performed with absolute alcohol. It was left for 5 minutes.
6. Some specimens showed the signs of collapsing. Then they were treated with the help of gentle massage and each one was stretched.

(3) Mounting of Thrips

Mounting was an essential procedure after the collection of insect’s fauna. It was necessary, because, if the collected insects were not mounted immediately, they could become hard.

Before the procedure of Mounting, we relaxed such sampled insects. Relaxation was done in a wide mouth airtight jar with moist sand. Blotting paper was spread as a cover over the mouth of the jar.

Thrips were placed in a solution prepared with alcohol (10 parts); glycerine (1 part) and 1 part of acetic acid mixture. It helped distend the bodies of the most of the thrips. In this manner their body parts keep supplied.

Such prepared specimens were stored until they could be mounted on microscopic slides. Stored specimens were kept in the dark and preferably at temperature well below $0^\circ$C to prevent the loss of their integument colour.

3.9 Research Methodology for Cultivation of Chilli Plants

Before the cultivation of sampled chilli plants we prepared the soil with natural minerals and without any use of pesticides so that the growth of all chilli seedlings picked up from different areas could be seen in healthy environment. The Net House was naturally ventilated and climatically controlled. It was made free from weeds and grass at regular intervals so that the necessary minerals for the growth of chilli plants might not be wasted. In this manner, temperature, humidity, light and intensity of soil media with proper irrigation were kept suitable for the growth of the chilli plants.

In order to facilitate our research objectives, and to obtain the pure yield production of chilli, *Capsicum annuum* under established experimental
net house conditions, chilli seedlings transplanted in sets of microplot. In total, seven experimental net houses were prepared for our controlled experiments.

We collected certain sample plants from different nurseries and transplanted them in the experimental microplots in order to find out results over damage on chilli plants with the help of our biological experiments.

Each nethouse was in size of 3x2 m with 4.5 ft height of the net. Chilli plants (*Capsicum annuum*) were transplanted on first week of January 2015 and first week of July in all micro-plots of the net house respectively. However, the cultivation of sampled plants have been arranged in a systematic linear fashion. Each plant was separated from the other at the distance of about 40-50 centimetres. The distance was maintained from row-to-row and plant-to-plant.

**Note:** Seedling plants were purchased from Krishna Nursery and Dev Nursery at G.T. Road, Aligarh.

3.10 Biological Controlled Experiments of Thrips Under Net House Conditions

The evaluation of our research was done by the process of rearing. Biological controlled experiments were done under seven net house of 21 microplots. These microplots were covered by nylon net. Each biological treatment was replicated at three times with the help of selected predators and parasitoids. Each experiment was performed till the population of thrips
reached at Economic Threshold Level (ETL). In this manner, we calculated the mortality in the experimental units.

In our experiments, the possibilities of use the predator; *Amblyseius cucumeris* and *Macrotacheliella nigra* and the parasitoids, *Thripobius semiluteus* and *Ceranisus menes* for the biological management of thrips on chill plants under net house conditions.

Figure 3.15: Prepared Net House for Biological Controlled Experiments

**Chapter 3: Research Methodology**

*Number of Figures: 15*
*Number of Charts: 0*
*Number of Tables: 0*