CHAPTER - II

MATERIAL METHODS AND PROCEDURE OF STUDY
The materials & methodology; in the present investigation have been made under the following steps. At first survey of different places of Sagar District, Sagar University Botanical Garden, Pathariya Jatt, Rehli, Deori, Banda, Rahatgarh, Bina & Agricultural field area of Sagar.

To begin with studies on the life history of different species, either the freshly laid eggs along with egg laying host/s or the larvae along with their host plant/s or both were brought to the laboratory for further rearing. Having obtained requisite field experience on certain behavioural aspects of butterflies and moths, one can clearly make out as to which of the female tends to oviposit, while flying around/nearer to her egg laying host/s. One or two such ovipositing females representing different species were also collected for the purpose of authentic identification. Such individuals were killed, pinned, stretched and preserved in air tight wooden insect boxes. The immature stages particularly the eggs, collected on different dates during the tenure of this research problem were placed in different sized petridishes (7.5 x 2.5 cm, 5x2 cm, 9x3.5 cm), which were sterilized before use. As per practice, some water soaked cotton covered with a piece of blotting paper was placed at the floor of each petridish before shifting the eggs. The rearing was done in a large flight cage/insectary (size, 4'x4'x4') and insect bottle are used in butterfly reared installed in an open space of the Botanical glass-house adjoining the Department of Botany and Prof. R.S. Saini's, Agricultural fram house in large Insect-cage. In a way, the insectary has almost the normal field conditions. Each petridish containing the developing stages was examined twice/thrice a day in order to make observations on aspects like incubation period, hatching, larval feeding, larval instars, larval ec dysis, larval duration, prepupation/pupation, larvo puual parasitism and the emergence of the adults etc. The measurements of different immature stages i.e., the eggs, larvae and pupae have also been taken. It may also be added that except for smaller larvae of lycaenids, the last instar larvae of other species were shifted in to portable insect cages of different sizes (8"x8"x8", 12"x12"x12") subsequently, the freshly emerged adults were released in the flight cage in order to ensure whether they mate or do not do so in captivity. Except Anaphaesis aurota (Fabricius) (Pieridae). none of the species mated in
captivity. However, it did not lay eggs on the egg laying host, capparis sepiaria (Linn.) (Capparaceae), provided in the cage.

Apart from this, observations such as the ovipositional behaviour and egg laying, patrolling, territorial behaviour, mating, courtship, mud puddling, predation, myrmecophily, nectral and larval food plants were recorded in the natural habitat in different areas of Sagar Region/localities besides head quarters at Sagar from-2001 to 2003. Before according observations, the original reference and the common name of the species have been given. The photographs of the immature Stages and the adults of all the Lepidopteran: butterfly thirty species and moth eighteen species have been studied:

**Thirty butterfly species i.e.:** Eurema (Terias) hecabe (Linnaeus), Catopsilia pyranthe (Linnaeus), Catopsilia pomona pomona (Fabricius), Delias eucharis Drury, Cepora nerrisa (Fabricius), Pieris (Artogeia) Canidia (Sparaman), Pieris (brassicae) nepalensis Gray (Pieridae); Papilio (princeps) demoleus Linnaeus, Papilio polytes Linnaeus, Graphium agamemnon, Linnaeus, Pachliopta hector Linnaeus (Papilionidae); Danaus (Anosia) Chrysippus (Linnaeus), Danaus genutia (plexippus) cramor, Hypolimnas missipus Linnaeus, Junonia lemonias Linnaeus, Ariadne merione cramor (Nymphalidae), Euploea core cramor Great eggfly, Tirumala limniace cramor, Melanitisleda cramor, Junonia hierta Fabricius, Junonia orithyia Linnaeus, Charaxes sp. Nymphalidae, Pelopidas mathias mathias (Fabricius) Hesperiidae; Zizeeria karsandra Moore, Lampides boeticus Linnaeus, Freyeria putli (Kollar), Pseudo Zizeeria maha (Killer), Chilades lajus lajus (cramor), Tarucus alteratus Moore (Lycaenidae) of Sagar Division and Eighteen Moths species i.e., Helicoverpa armigera (Hubner), Agrotis ypsilon (Rott.), Achaea janata Linnaeus (Noctuidae); Symtomis sprearis Fabricius [Amatidae (Symtomidae)]; Sylepta derogata Fabricius, Leucinodes arbonalis Guen. Chilopartellus) Zonellus) Swinhoe (Pyralidae); Hyblaea puera cramor (Hyblaeidae); Pectinophora gossypiella sound. (Gelechiidae); Achaerontia styx Westwood, Herse Convolvuli Fabricius (Sphingidae), Macroglossum stellatorum, Daphnis nerii, Nephele didyma Fabricius (Sphingidae); Eumeta crameri Westwood (psychidae); Cossus-cossus (Cossoidae); Eueterote fabia Linn. (Bobycidae); Antheraea paphia Linnaeus (Saturniidae) of Sagar Division (M.P.).
Collection of the Material:

To achieve the objectives of the present research proposal, the first step involves the collection of the Butterfly Lepidopterans and moth fauna referable to the super families of Butterflies as:

1. Papilionoidea
2. Hesperioidea

and referable to the super families of Moths as:

1. Noctuoidea
2. Pyraloidae
3. Gelechioidea
4. Saturuioidae
5. Bombycoidea
6. Sphinxoidea
7. Cosoidea
8. Psychoidea

From the Sagar division (M.P.) In India.

Accordingly, the survey tours of varied duration were conducted in the area, under study. It may be mentioned here that the Sagar as the Range of Satpudas hilly are. According to an estimate, the altitude of the Sagar water box hilly range goes up to 1960 feet above. The portion of this range selected for present studies comprises the plain and hilly are of the Sagar Division. Localities such as Sagar V.V. Botanical garden, campus area, Pathariya Jatt agricultural and forest, Rehli, Deori & Banda. In the respective area were surveyed between 2001 to 2003. During the premonsoon, monsoon and post monsoon seasons for collection of the material, presently studied.

In view of nocturnal behaviour of the moths, workers such as common (1959), Common and Upton (1964), Heath (1976) and Fry and Waring (1996) have suggested various types of traps and techniques for their collection. Accordingly, collection of the material required for present studies was done with the help of portable light trap/s (Plate ------). In this model of the light trap, the source of light to attract the moths was a 125 w Mercury Vapour lamp fixed within a Steel funnel (diameter top 30 cm, bottom 6 cm; height 30 cm). The funnel in fitted with baffle plates so that the moths visiting the light get trapped around the lamp. The funnel is fixed on the top of a collecting
chamber (30 cm x 30 cm x 12 cm) fitted with a sliding collecting tray (29 cm x 29 cm). Before putting the trap into operation, the collecting chamber was charged with 1, 1, 2, 2, tetrachloroethane as a killing agent for the moths. The petridish containing cotton soaked in aforesaid charge was placed in one of the corners of the tray. The traps were installed near the ground level amongst the bushes for the collection of micrws, who are generally weak fliers. In addition to the light traps being used for collection, a MV lamp was hung along a white cloth sheet secured to a wall or directly over a plain white wall, as also recommended by Fry and Waring (1996). The sitting posture and flight behaviour of certain moths help their identification atleast upto the family level in many instances. Some of the specimens were collected singly in the glass killing tubes of various sizes (2 cm x 7 cm to 5 cm x 15 cm) from the light sources fixed at places such as petrol pump stations, public buildings and Rest Houses around the locatity being visited. Some of the individuals were collected during the day time with the help of an insect collection net (Diameter 30 cm, handle length 90 cm, bag depth 80 cm.).

**Spreading of the Specimens and Their Preservation**

Workers like Amsel (1935), Holland (1937), Lindquist (1956), Hodges (1958), Tagested (1974), Zimmerman (1978), Nielson (1980), Sokoloff (1980), Mikkola (1986) and Landry and Landry (1994) have suggested certain ways and means to spread the specimens before they are examined for taxonomic purposes. As such, the method involves pinning each specimen through the mesothorax by drolling the same in dorsal position with the help of forceps. According to size of the specimens, the entemologi pins of different sizes (minuten headless pins).

Maximum & Minimum Temp. and relative humidity majored month to month humidity majored by hygrometers was used and for the study of population the marking methods capture and recapture butterflies and moths were applied.