3. MATERIALS AND METHODS

The whitegurbs are polyphagous both in grub and adult stage and they feed on a wide variety of cultivated and uncultivated plants. Most of the species are crepuscular or nocturnal, rarely seen by casual observer except when the beetles are attracted to light. The adults become active, fly about during the night and feed on the foliage of fruit/forest trees (Chandel and Kashyap 1997). The grubs live concealed in soil and almost all field crops grown during rainy season are damaged. Accordingly, the field survey work related to the present study was started in March 2011 to collect different stages of phytophagous whitegrubs. The survey work continued for about two years in Himachal Pradesh. The culture of predominant species was maintained separately in the whitegrub laboratory, Department of Entomology, CSK HPKV, Palampur lattitude N $32^0$05.985′ and longitude E $76^0$32.831′ at 1238 m amsl to record observations on biology and morphometrics of different species of whitegrubs.

3.1 Collection of sacarabaeid beetles from different parts of Himachal Pradesh

The beetles of phytophagous whitegrubs were collected on light trap and directly from host trees at night. Data on latitude, longitude were recorded using Global Positioning System (GPSmap 76 CS x, Garmin Make) for each location.

3.1.1 Collection of adult beetles on light trap: The population of sacarabaeid beetles was monitored through UV light trap installed in six different districts in zone I (sub-tropical sub-mountane and low hills), zone II (sub-temperate sub humid mid hills), zone III (wet-temperate high hills) and zone IV (dry-temperate high hills and cold deserts) of Himachal Pradesh during 2011 and 2012. All the study areas were either fruit orchards or farmland with different cropping patterns. The details of study sites are given in table 3.1. The light traps were used for about four months and beetles were monitored once a week. The UV light traps used in the present study were supplied by M/S Himalayan Industries, Baddi, Solan, Himachal Pradesh.

The body of the light trap was made of red coloured PVC plastic. The plastic funnel was 25 cm in height having top diameter of 30 cm and bottom diameter of 5-6 cm. The rain shed cone for the protection of bulb was fixed about 17 cm above the funnel with the help of three white metal sheets. The diameter of the rain shed cone was 20 cm.

The special features of this trap are the baffles, which are three in number and placed at a uniform distance of 10 cm around the circumference of funnel. The baffles are white in colour, made of M.S. sheet, 30 cm long and 11 cm wide. The baffles are fixed in such a way so that light rays are emitted uniformly in all the directions without any interference; however, the beetles attracted to light collide with these baffles and fall into the trap. An efficient bag was attached to the bottom of this funnel. The light source consisted of hard glass bulb with choke of copper wire. The capacity of bulb was 120 watt and it was with UV radiation with visible spectrum having bluish light (Plate 3.1).

These light traps were installed at 8 locations and there was one trap at each location. The light trap was placed in the centre of the field at a height of about 10 feet and operated from 7:00 PM to 11:00 PM to attract chafer beetles, being positively heliotactic in nature. The trapped beetles were collected and separated species-wise and cummulative count of each species was determined at each location. These beetles were grouped on the basis of relative abundance and frequency values for the purpose of investigating the relative importance of the different species of whitegrubs. The frequency data were grouped into four categories as suggested by Tischler (1949) and the abundance values were grouped into two as suggested by Weis-Fogh (1947-48).

Table 3.1 Location details for light trap studies in Himachal Pradesh, 2011 and 2012

District	Location	Zone	Latitude	Longitude	Altitude(m)	Crop habitat
Kangra	Palampur	II	N 32^0, 05.666′	E 76^0, 32.781′	1222	*Toon*, peach, pear
Kullu	Seobagh	II	N 32^0, 02.958′	E 76^0, 37.533′	1327	Apple , pear
	Dallash	III	N 31^0, 23.036′	E 77^0, 26.024′	2020	Apple, pear
Sirmaur	Kwagdhar	III	N 30^0, 45.409′	E 77^0, 09.235′	1774	Apple, pear, peach, rose
	Kheradhar	III	N 30^0, 50.035′	E 77^0, 20.634′	2032	Apple, walnut
Shimla	Shillaroo	III	N 31^0, 12.409′	E 77^0, 25.462′	2479	Apple
Kinnaur	Reckong Peo	IV	N 31^0, 31.348′	E 77^0, 47.856′	2117	Apple, pear, walnut
Chamba	Bharmour	IV	N 32^0, 26.505′	E 76^0, 31.949′	2169	Apple, pear

3.1.2 Adult abundance in flight trees: Adult surveys to determine species occurrence and relative abundance were conducted in the nine important fruit growing areas of Himachal Pradesh (Table 3.2) during the rainy seasons of 2011 and 2012. In 2011, beetles were collected at locations near Shillaroo, Palampur, Kullu, Seobagh, Dallash,

Plate 3.1: UV light trap installed at Palampur (a), and UV light trap in operation at Kwagdhar (b)

Reckong Peo, Sangla, Nauni, Kwagdhar, Kheradhar, Bharmour and Kheri, from areas mostly known to be endemic to whitegrubs (Fig. 3.1). In 2012, collections were repeated at all the locations. Generally the sacarabaeid beetles are nocturnal in behaviour and come out of soil at dusk. Chandel et al. (1995) reported that beetles come out of soil around 7:50 PM at light intensity of 3 lux and settle on host trees for mating and feeding. Therefore, it was ensured to reach at the collection site by 7:30 PM on the day of collection. After suitable rainfall events, representative samples of beetles were collected during March-September from trees, including apple, apricot, walnut, peach, pear, *Ficus*, *toon*, *khirak*, citrus, wheat, grasses, guava, *Robinia*, *Grewia*, *kachnar*, wildrose, paddy and maize. In these areas, trees 3-5 m high were commonly selected. Five plants were marked at random in each orchard/farm in all the surveyed localities and three branches of almost equal length were selected. The beetles were collected between 18:30-19:30 hours using a powerful flash light. This collection was done during the peak emergence months of March-September for different species followed by fortnightly observations during the entire monsoon season. A simple umbrella method was used for collecting beetles from trees as suggested by Chandel et al. (1997). On each sampling date, an umbrella was spread below each tree and the branches were shaken one by one to dislodge the beetles. The dislodged beetles were handpicked and transferred to metal cages containing shoots of host trees on which collection was done.

All the beetles collected at various locations were brought into the laboratory and they were sorted out by species level to compile a list of sacarabaeid fauna at each location. For the purpose of this survey, any tree on which scarabaeid adults could be collected feeding and/or mating was considered to be a host tree. Trees in and around a selected location were sampled in a semi-systematic manner ensuring that all the tree species at the locality were examined.

At some locations, the survey was extended with the assistance of staff working in Agriculture/Horticulture departments. The process involved supplying collection equipments and the provision of demonstrations and detailed collection instructions to the concerned persons.

Table 3.2 Description of sampling locations for beetles on host trees during 2011 and 2012

District	Area surveyed	Zone	Latitude	Longitude	Altitude (m amsl)
Kangra	Palampur	II	N 32^0, 05.666′	E 76^0, 32.781′	1222
	Panchrukhi	II	N 32^0,06.350′	E 076^0,57.980′	1078
Kullu	Seobagh	II	N 32^0, 02.958′	E 76^0, 37.533′	1327
	Dallash	III	N 31^0, 23.036′	E 77^0, 26.024′	2020
Shimla	Shillaroo	III	N 31^0, 12.409′	E 77^0, 25.462′	2479
Kinnaur	Reckong Peo	IV	N 31^0, 31.348′	E 77^0, 47.856′	2117
Solan	Nauni	III	N 30^0, 51.818′	E 77^0, 09.105′	1255
Sirmaur	Kwagdhar	III	N 30^0, 45.409′	E 77^0, 09.235′	1774
	Kheradhar	III	N 30^0, 50.035′	E 77, 20.634′	2032
Chamba	Bharmour	IV	N 32^0, 26.505′	E 76^0, 31.949′	2169
Bilaspur	Berthin	I	N 31^0,12.310′	E 76^0,23.458′	461
Hamirpur	Kheri	I	N 31^0, 53.299′	E 76^0, 35.759′	456
	Jahu (Bhararei)	I	N 31^0,38.630′	E 76^0,41.062′	838
Mandi	Jogindernagar	II	N 31^0,59.248′	E 76^0,49.362′	1465
	Karsog	II	N 31^0,26.599′	E 77^0,04.599′	1860
Una	Rampur	I	N 31^0,27.301’	E 076^0,15.541’	393

3.2 Identification of beetles

The sacarb adults collected during the survey and the adults emerging from larval collections were identified to species level based on the keys and character lists given by Veeresh (1977), Mittal and Pajni (1977), Khan and Ghai (1982) and Ahrens (2005). The identity of adult beetles was confirmed by Dr. V.V. Ramamurthy, Indian Agriculture Research Institute, New Delhi. Some of the samples were compared with sacarabaeid collection available in Museum of Forest Research Institute, Dehradun. Representative specimens from the survey reported here are lodged with Dr. V.V. Ramamurthy, Division of Entomology, Indian Agricultural Research Institute, Pusa, New Delhi, India.

3.3 Determination of diversity indices

Richness (number of species), abundance (number of Individuals) and four diversity measures were used in this study. The diversity indices assume that individuals are randomly sampled from an infinitely large population. The Shannon index (H') explains the evenness of the abundance of species, while the Simpson’s index of diversity (D) is less sensitive to species richness but more sensitive to the most abundant species, (Price 2004). Pielou’s evenness index (J') explains the evenness of allotment of individuals among the species. Diversity indices were determined from all the information gathered during the period of study on each site by using the following equations, (Krebs 2001).

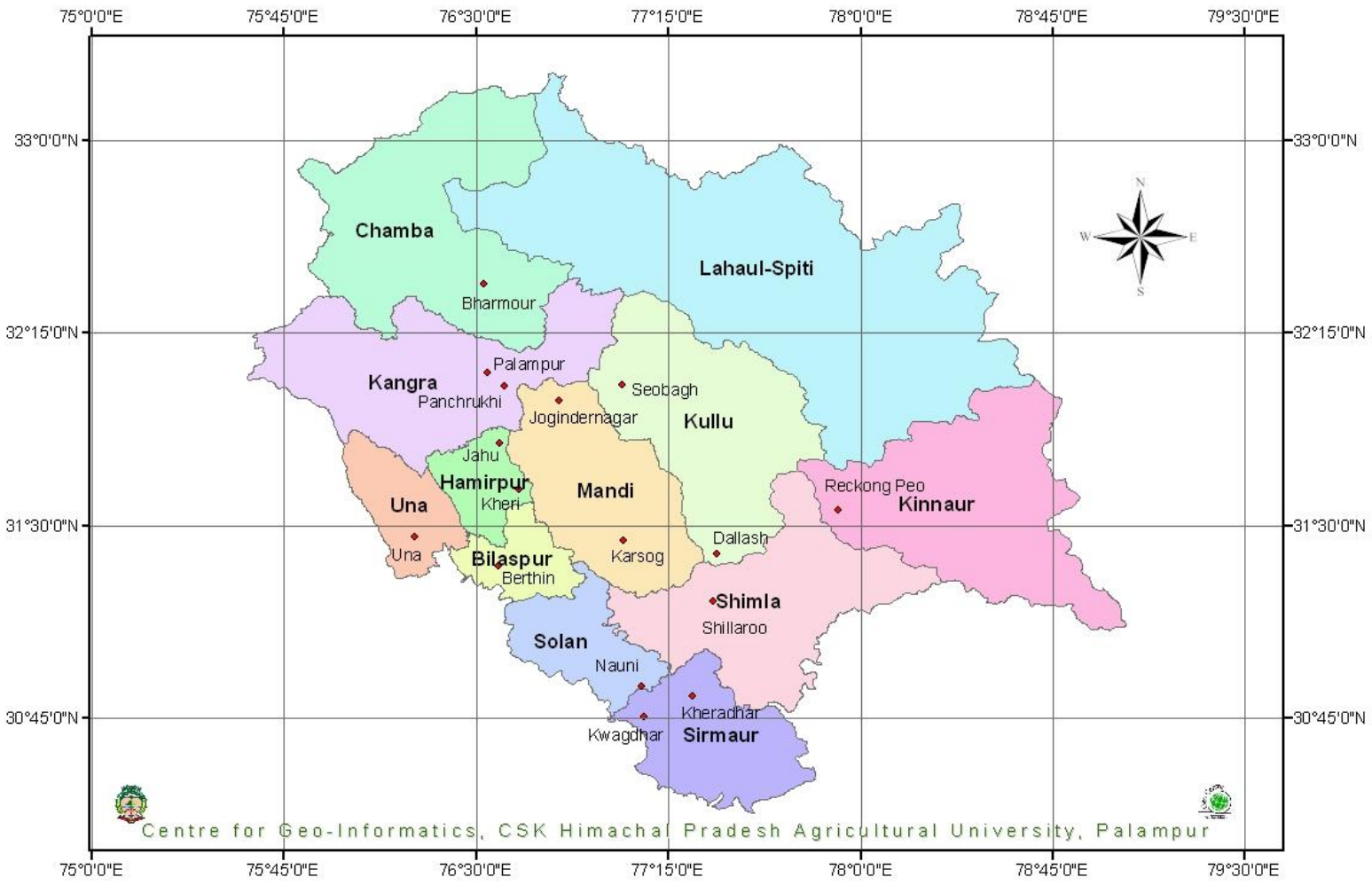

Fig. 3.1: Map showing sampling locations for beetles on light trap and host trees in Himachal Pradesh

i) Shannon index

$$H' = - \sum_{i=1}^{s} (p_i) \quad (\log_2 p_i)$$

ii) Simpson's index of diversity

$$D = 1- \sum_{i=1}^{s} (p_i)^2$$

iii) Simpson's reciprocal index= 1/D

iv) Pielou's evenness index (J')

$$J' = H'/ H_{max}$$

Where

H'= Shannon diversity index

p_i= Proportion of total sample belonging to the i^{th} species.

S= Number of species.

∑= Sum from species 1 to species S

D= Simpson's index of diversity.

N= Total percentage cover or total number of organisms.

n= Percentage cover of a species or number of organisms of a species

J'= Evenness of allotment of individuals among the species.

H_{max} = Maximum species diversity (H') = Log_2S

3.4 Estimation of damage relationship of beetles on different trees

The scarabaeid beetles generally defoliate the leaves of wide range of fruit and forest trees. To study the extent of leaf defoliation, randomly selected 100 leaves of a selected branch at a plant were monitored for healthy and damaged leaves and per cent defoliation was calculated. Data were generated from 10 most preferred host plant at each location. To calculate the leaf injury index, the per cent defoliation was visually scored using 1-5 scale as shown below.

Score	Rate of defoliation (%)
1	1-20
2	21-40
3	41-60
4	61-80
5	>80

The leaf injury index was calculated using the following formula

$$\text{Leaf injury index (I)} = \frac{\sum n}{N}$$

Where

n= Per cent defoliation in each sample

N= Total number of samples

3.5 Estimation of whitegrub population in soil and their extent of infestation

The locations selected for beetle collection during 2011 and 2012 were also surveyed for larvae during September-October of 2011 and 2012. Some additional locations were also covered (Table 3.3, Fig. 3.2). In crops like maize, peas, beans, okra tomato, capsicum, cabbage, the wilting plants and the plants that had died prematurely were uprooted and the soil around these plants was searched for larvae. In crops like potato, ginger or in fallow fields, sampling sites were selected randomly by throwing a one square feet quadrant over ones shoulder, with no two sampling pits overlapping. Pit sampling was done and a sample consisted of one cubic feet of soil, carefully dug by a shovel and searched for larval, pupal and adult stages. Eggs were too small thus too tedious to be sampled. Ten samples in each crop were taken during all the observations. Larvae were transferred to the laboratory to be reared through to the adult stage to facilitate identification because knowledge of adult-larval association was incomplete for whitegrub species occurring in Himachal Pradesh.

At the same time as larval samples were collected, data on damage were also assessed in different crops. The averages of all observations were worked out and plotted to draw conclusions. Association between the presence of whitegrubs and the incidence of plant damage was evaluated using correlation analysis in crops like potato, ginger, beans, maize, and cabbage.

3.6 Studies on the biology and morphometrics of important species of whitegrubs

Mating pairs of beetles were collected from field and brought to laboratory at Palampur in metal cages (15x10 cm^2). In the laboratory, groups of three pairs were placed in glass jars (15 cm diameter) half filled with mixture of moist sand, soil and farm yard manure in equal proportion. Tap water was added to keep soil damp. Freshly cut twigs of

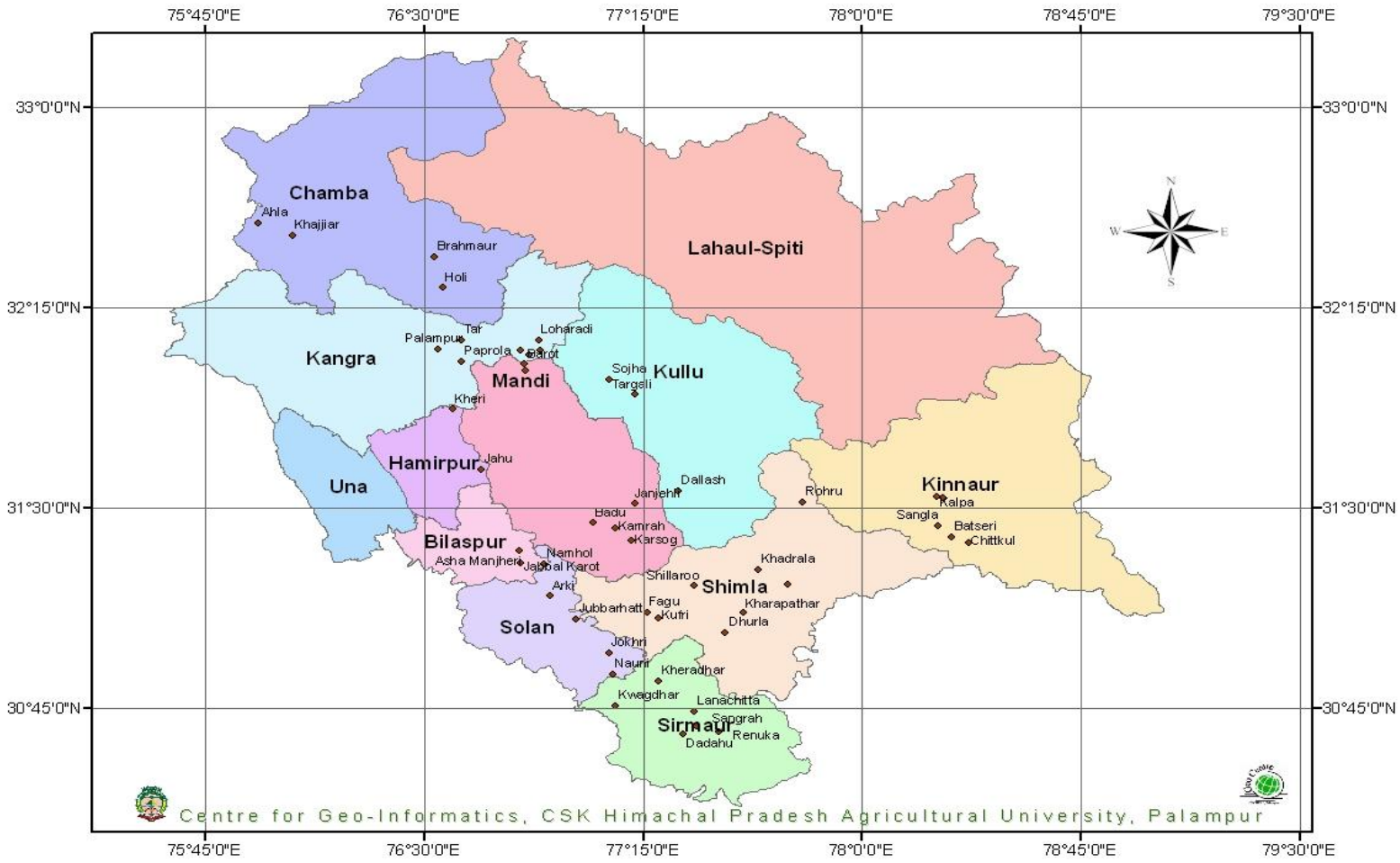

Fig. 3.2: Map showing sampling locations for whitegrubs in Himachal Pradesh

Table 3.3 Description of locations surveyed for whitegrubs in Himachal Pradesh

District	Location	Zone	Longitude	Latitude	Altitude (m amsl)	Crop habitat
Kangra	Palampur	II	N 32^0, 05.666′	E 76^0, 32.781′	1222	Cole crops
	Paprola	II	N 32^0, 02.956′	E 76^0, 37.531′	978	Cole crops, Okra, peas, brinjal, peas, potato
	Baragaon	III	N 31^0, 58.957′	E 77^0, 07.907′	2268	Potato, rajmash, cole crops
	Loharadi	II	N 32^0, 04.523′	E 76^0, 51508′	1948	Rajmash, Cole crops
	Kothi Kohad	III	-	-	-	Cole crops
	Kothi Swar	III	-	-	-	Cole crops
	Tar	III	N 30^0,50.939′	E 77^0,18.011′	2687	Potato
Kullu	Dallash	III	N 31^0, 23.036′	E 77^0, 26.024′	2020	Potato
	Sojha	III	N 31^0, 33.867′	E 77^0, 22.268′	2501	Cole crops, potato, maize
	Targali	II	-	-	-	Maize
Mandi	Barot	II	N 32^0, 02.647′	E 76, 50.415′	1847	Potato, rajmash, cole crops
	Janjehli	III	N 31^0, 30.864′	E 77^0, 13.307′	2100	Potato, peas
	Badu	II	-	-	-	Cole crops
	Karsog	II	N 31^0,26.599′	E 77^0,04.599′	1860	Potato
	Kamrah	III	-	-	-	Potato
	Phulladhar	III	-	-	-	Potato
Shimla	Shillaroo	III	N 31^0, 12.409′	E 77^0, 25.462′	2479	Potato
	Fagoo	III	N 31^0,05.187′	E 77^0,18.149′	2519	Potato
	Kufri	III	N 31^0,06.051′	E 77^0,15.812′	2561	Potato
	Kharapathar	III	N 31^0, 06.433′	E 77^0, 35.484′	2728	Potato
	Khadrala	III	N 31^0,16.145′	E 77^0, 38.746′	2831	Potato
	Rohru	III	N 31^0, 12.695′	E 77^0, 44.078′	1526	Potato, maize, peas
	Dhurla	III	N 31^0, 01.884′	E 77^0, 32.109′	2516	Potato
Kinnaur	Reckong Peo	IV	N 31^0, 31.348′	E 77^0, 47.856′	2117	Fruit orchards
	Sangla	IV	N31^0, 25.932′	E 78^0, 15.060′	2588	Potato, peas, carrot
	Kalpa	IV	N 31^0, 32.344′	E 78^0, 16.626′	2682	Rajmash,
	Chitkul	IV	N 31^0, 32.502′	E 78^0, 15.294′	3378	Peas
	Batseri	IV	N 31^0,23.384′	E 78^0,18.345′	2684	Apple, peas
Solan	Nauni	II	N 30^0, 51.818′	E 77^0, 09.105′	1255	Fruit orchards
	Jokahri	II	N 30^0, 57.887′	E 77^0,07.704′	1321	Capsicum, maize, potato, wheat
	Jubbar Hatti	II	-	-	-	Ginger
	Arki	II	-	-	-	Maize
	Kunihar	II	-	-	-	Maize
	Deothi	II	-	-	-	Cole crops
Sirmaur	Kwagdhar	III	N 30^0, 45.409′	E 77^0, 09.235′	1774	Apple, peach, pear,walnut
	Kheradhar	III	N 30^0, 50.035′	E 77, 20.634′	2032	Potato
	Lanachitta	II	N 30^0, 47.359′	E 77^0, 22.422′	1246	Ginger
	Sangrah	III	N 30^0, 40.815′	E 77^0, 25.845′	1575	Ginger
	Renuka	II	-	-	-	Ginger
	Deedag	III	-	-	-	Peas
	Kafota	II	-	-	-	Ginger
	Masu	II	-	-	-	Ginger
	Harlo	II	-	-	-	Ginger
	Dongi	II	-	-	-	Ginger
	Chereu	II	-	-	-	Ginger
Chamba	Bharmour	IV	N 32^0, 26.505′	E 76^0, 31.949′	2169	Potato, maize, rajmash
	Holi	IV	N 32^0, 19.492′	E 76^0, 33.750′	1879	Maize, rajmash
	Khajjiar	III	N 32^0, 32.042′	E 76^0, 32.298′	1946	Maize
	Ahla	III	-	-	-	Potato
Hamirpur	Kheri	II	N 31^0, 53.299′	E 76^0, 35.759′	436	Maize
	Jahu	I	N 31^0, 38.630′	E 76^0, 41.062′	838	Potato
Bilaspur	Namhol	II	-	-	-	Ginger
	Jabbal Karot	I	N 31^0, 20.258′	E 76^0, 49.523′	685	Ginger
	Asha manjheri	I	N 31^0, 17.460′	E 76^0, 49.796′	801	Ginger

their preferred hosts pressed into the soil were provided as food for adults and were replaced daily. Each glass jar was covered with a glass chimney and the upper grooved end of chimney was covered with muslin cloth using rubber band to prevent beetles from escaping Plate (3.2). The glass jars were left outside in laboratory and maintained at room temperature.

The soil was tipped out of glass jars every 1-2 days, and eggs were removed and counted. The same soil was used for about a month, and was examined every time it was tipped out of the jars. Eggs collected from oviposition jars were placed on moist soil in Petri dishes and left outside to incubate under the same conditions as adults. The eggs were checked each day until eclosion. After eclosion, hatched out larvae were removed from Petri dishes gently and placed in small cups containing a mixture in which maize seedlings were growing or had recently been sown (Plate 3.3). Each larva was placed in a single cup. The maize was resown every two weeks and the soil maintained in a moist condition by careful watering. Each cup was filled with about 100 g of soil. Second and third instar grubs were maintained on potato tubers and these were given *ad libitum*. Development and survival was checked by emptying out the soil and examining the contents every week except during the prolonged resting periods at the end of third instars, when it was done almost monthly. Development inside the earthen cell was followed by viewing either through a small hole or, when the cell had been constructed adjacent to the glass, through the side of the jar. When numbers allowed, sequential examples of larvae of known age were killed and preserved in alcohol during the development of a batch of larvae.

Measurements on head capsule and body length were taken from sequentially killed larvae during development from newly hatched larvae to pupation. The grubs were observed regularly and different larval instars were distinguished on the basis of head capsule width as suggested by Chandel et al. (1995). Newly formed grubs were used to measure their size in each instar (n = 10). Data on head capsule width were recorded using Olympus SZ-16 image analysis system (Progres Jenoptik). The size of larvae, pupae and adults was measured using digital Vernier Caliper (0-150 mm, Aerospace make). The rasteral patterns of larvae were drawn with the help of Camera Lucida.

Plate 3.2: Glass jars and chimney sets used for mating and feeding of beetles

Plate 3.3: Paper cups used for rearing of grubs

3.7 Studies on male and female genitalia of beetles

After night collection, the beetles of different species were brought to laboratory in metal cages and sexing was done. In some cases, male and female beetles were separated at the time of mating and the beetles were preserved sex-wise in separate plastic containers. Veeresh (1977) reported that sex in sacarabaeid beetles can be distinguished by pressing the abdominal tip between fingers applied laterally in case of fresh specimens, the internal genitalia comes out as soft whitish fleshy mass with the paramere tips visible through the anal end of the male beetles and not in the case of the females. The same method was adopted here to distinguish the sexes as shown in plate 3.4. The female genitalia were pulled out through the opening between the last dorsal and ventral segments. The male genitalia were pulled out through the last abdominal segment. After dissection, the genitalia were potashed in 10 per cent KOH solution for about 20-30 minutes and then cleared-off from any extra tissues. The female and male genitalia were preserved in clove oil and 70 per cent ethanol, respectively. After that, studies were made under a stereo zoom microscope (Olympus SZ-16; Software, Progres Jenoptik) and line diagrams drawn with the help of Camera Lucida by keeping them in 70 per cent alcohol.

Plate 3.4: Sexing of beetles a) Male beetles b) Female beetles