Materials and Methods:

The first pre-requisite for any taxonomic study is the collection of the representative samples. No systematic work can be deemed as complete unless it includes the study of a large number of specimens collected from a wide range of study sites.

4.1 Collection

The work of collection was undertaken in Kumaun hills from 2007 and was carried out regularly for three years, covering all important Citrus growing areas of the five districts, both in the hills and in the low lying valleys, with particular attention to the sub-montane tracts, where most of the commercial Citrus plantations occur. The collection of wild specimens from the forests uninhabited by human beings was done mostly with the help of the local inhabitants, tribes, resource persons and the Department of Forest. The work was executed in a very thorough manner and there is hardly any important Citrus track in the province situated up to 3000 m, which was not visited in the quest for the Citrus species.

The collection of different specimens consisted of gathering both mature fruits and inflorescence with leaves. Seedlings and wherever available, clonal progenies of different varieties were also collected. For each specimen a minimum of three to five fruits were dissected and at least one herbarium sheets embodying inflorescence and leaves were also preserved. For detailed observational study a nursery was maintained representing seedlings or clonal progenies of the specimen of interest. During collection no attempt was made to eliminate the replicas unless the similarity was very obvious. This cautious handling of the materials, even at the risk of enormous multiplication of work, was adopted to minimize the chance of any new type or form slipping out. The
enormity of the work may be judged by the fact that there are 448 accessions of different species that have been studied.

4.2 Characterization
Characterization of the collected material was systematically recorded and the passport data of each and every accession prepared as per the *Descriptor for Citrus* developed by International Plant Genetic Resources Institute IPGRI (Anonymous, 1999). However, the guidelines of the descriptor is based on several vegetative and reproductive parts of the *Citrus* but in the present thesis work only description of flower, fruit, segment, pulp and seed (starting from 7.3 to 7.7 headings, mentioned in appendix-1) has been incorporated. Standard colour chart, e.g. Royal Horticultural Society Colour Chart was used for all ungraded colour characters.

4.3 Preparation of the herbarium
Preparation of the herbarium remains the fundamental aspect of the study, which has been performed through different levels as follows.

4.3.1 Collection of the herbarium specimen
The healthy and disease free specimens with at least 3 replicates of the same plant (flowering twigs, wherever possible) were collected with the help of secatier. Simultaneously the string tag was used with accession number. The self developed field press having a pair of plywood (12"×17") and four nut-bolts with newspaper blotters was used to press the specimens. The press was locked up for about 24 hours (sweating period). The field notebook as an indispensable item was filled on the spot to record the data, such as date, place, locality, habitat, elevation, local name, uses and accession number etc. Accession numbers were arranged in a numerical series starting with 001 and up to so on for the final one specimen selected out of three replicates.
4.3.2 Drying
Specimens were dried as rapidly as possible to get the best results. By the usual process of drying, the press containing the specimens was placed in the sunlight. After 24 hours the press was opened, and specimens were placed in the fresh blotters. The press along with the fresh blotters was again bound tightly. The wet blotters were dried in the sunlight for reuse and for consecutive next three days the blotters were changed daily, until the specimens were completely dried. For the collection and drying of the specimens, very low humidity period, mid-winter to early summer days were selected.

4.3.3 Poisoning and mounting
The dried specimens were dipped in a 2 percent saturated solution of mercuric chloride in ethyl alcohol to prevent the infection of fungi, insects etc. The poisoned solution was poured in to a glass trey and each specimen, before mounting, was dipped in it, returned to its blotter, and dried between blotters for 24 hours. Following this, it became ready for mounting. Good quality glue was applied to the back of the specimens for affixing them on a standard size of herbarium sheet 11"×14". Cotton thread was used for stitching to hold the woody part of specimen. Loose parts (e.g. seed and flower) were pasted on the same sheet.
(A) Drying of the specimens and pressing again with fresh blotters.

(B) The self developed field press having a pair of plywood (12" × 17") with nut-bolts.

PLATE- 4.1
4.3.4 **Labeling**
A label of 6.5×10.5 cm, Jones and Luchsinger (1987) dimension was pasted on the lower right side of the herbarium sheet. Label contains the definite information about the specimen pasted on the herbarium sheet. The following information was incorporated on the label:
1. Heading, indicating the name of college, university, along with the name of state and country.
2. Family name
3. Botanical name, *i.e.* name of genus, species and variety along with author citation.
4. Locality, indicating the place of collection with its latitude, longitude and altitude from the sea level.
5. Habitat, indicating cultivated, wild or semi wild.
6. Date of collection.
7. Collector’s name.
8. Accession number.
9. Local or vernacular name.
10. Additional information.

4.4 **Illustrations**
Illustrations are an important part of any taxonomical work. A single picture may tell more than a page or indeed several pages of prints. This explains the reason for presenting the numerous illustrations and photographs in support of the descriptive details of the different species and varieties. All the pictorial representations have been drawn to the natural scale.

4.5 **Collection and germination of the seed**
The seed germplasm of rare and endangered species like *Citrus medica* L. and other valuable ones were collected from the healthy fruits. The seeds were carefully kept and washed in a non-metallic pot and subsequently dried in shade. A nursery was developed at Didihat, 1650 m. Here the seedlings of selected species were developed traditionally, as well as in polybags.

**4.6 Description**

All the representative *Citrus* plants have been studied with reference to numerous important taxonomic features, comprising of characters of leaves and flowers, as also the external and internal characters of the fruits. While recording the diagnostic details of leaves, flowers and fruits, only fresh samples representing a fair average of the variety under study were considered, avoiding variable extremes. This was necessary as the variation in size of leaves, flowers and fruits in *Citrus* are quite considerable. This is particularly so in the case of those species which flower and fruit more than once in a year e.g. *Citrus aurantifolia* (Christm & Panz.) Swingle. The difference in size between the big summer leaves and the small winter ones, between the flowers and fruits of the normal season and those of the ‘off season’ is amazingly great. Even amongst the fruits of the same tree of the same season the extent of variation in size and juice content is observed to be very marked.

All the taxonomic details and other information (e.g. locality, indicating the place of collection with its latitude, longitude, altitude, local or vernacular name and uses etc.) were systematically recorded as a rough draft of description. Apart from recording the taxonomical details, representative fruit samples of most of the *Citrus* species were also
(A) The storage of washed and dried seed germplasm in non metallic pots.

(B) A herbarium sheet of 11"×14" with poisoned specimen.

(C) Seedlings of different *Citrus* species developed in traditional raise bed.

PLATE- 4.2
dissected and the details of flower, fruit, segment, pulp and seed were recorded in the *Descriptor of Citrus* as per IPGRI (Anonymous, 1999). After identification of the species, an average of the data from the descriptor was taken for the fair writing under a species. This has ensured more or less uniform and standardized descriptions and facilitated an easy comparison of the characters of different species. The identification of different species was done by critically studying almost all available literature on the subject and comparing the herbarium sheets and illustrations with the identified type specimens as preserved in the herbaria of National Botanical Research Institute (N.B.R.I.), Lucknow.

4.7 Polygraph modeling of the target species:
For the objective representation of species diversity among *Citrus* groups, the polygraph method has been adopted, devised by Hutchinson (1936) and elegantly utilized in understanding *Xanthium* and *Empetrum* Love and Nadeau (1961). This technique has been applied amongst the total species encountered (cultivated and wild) to discover the seemingly similar species within the group and also to discern how large the dissimilarities are.
4.8 The key used for the specimen identification (adapted from Swingle 1943).

**Key to the subgenera**

**A.** Pulp-vesicles free from acrid oil-droplets; petiole not or nearly winged; flower large or small, white or tinged; stamen polyadelphous…

(subgenus *Citrus*)

**AA.** Pulp-vesicles contains acrid oil-droplets; petioles broadly winged; wings as broad as blades; stamen usually free…

(subgenus *Pepeda*)

**Subgenus Citrus, key to the species**

**A.** Petiole absolutely wingless, not articulated; flower tinged, staminate or bisexual; fruit large to very large, rind very thick, carotty, sweet,…

*C. medica*

**AA.** Petiole marginate or narrow to broadly winged, articulate at base; fruit not as above…

**B.** Petiole narrowly marginate…

**C, D, E**

**BB.** Petiole broadly winged…

**F**

**C.** Flowering all the year round, tinged, bisexual; fruits medium sized, smooth, ovoid-oblong, adherence of carpel strong, mamillate; pulp vesicle pale green to yellowish, sour; rind adherence strong…

*C. limon*

**CC.** Flowering once annually, tinged, bisexual; fruit medium sized, very warty bumpy, oblong to somewhat spherical, carpel easily separable; pulp vesicle pale yellow, sour, rind adherence medium…

*C. jambhiri*

**D.** Flower purplish tinged; juice intensely sour; fruit medium, ovate-oblong; rind adherence medium to strong, thick, spongy, sweet; pulp vesicle orange…

*C. karna*
DD. Flower pure white; juice usually sweet; fruit medium, oblate globose or pyriform; rind loosely attached, baggy, thin, pulp vesicle orange..............................................C. reticulata

E. Juice insipid sweet (acidless); petiole wingless, short; flower pure white, pulp vesicle white, insipid sweet (acidless), aroma distinct; seed few, chalazal spot light yellow..........................C. limetta

EE. Juice sour; petiole with rudimentary wing; flowers white or slightly tinged outside; pulp vesicles acid, bitter principle not marked, not easily separable, white or pale yellow..............................................C. megaloxycarpa

F. Petiole with moderately large to spatulate wing, articulated.................................................................G, H

G. Aroma of leaf distinct (typical bitter orange); petiole broadly winged; pulp vesicle orange tinged, very sour; peel deep orange to scarlet red.............................................C. aurantium

GG. Aroma of leaf distinctly different from that of bitter orange; petiole narrowly winged; pulp vesicle yellow to orange, sweet; peel yellow to orange ..............................................C. sinensis

H. Fruit small; pulp vesicle slender, fusiform, greenish white intensely acidic and sour; seed small; peel very thin greenish yellow to yellow; petiole obovate to spatulate; flowers pure white, all the year around .................................................................C. aurantifolia

HH. Fruit very large, either globose or oblate to pyriform; petiole broadly winged, spatulate or obcordate; flower large, greenish white; pulp vesicle sub acid, easily separable, pale green to pinkish .................................................................C. maxima