Chapter 3

MATERIALS AND METHODS

4- Dimension of conidia (length and breadth).

5- Presence or absence of fibrosin bodies in conidia.

6- Mode of germination of conidia and type of the germ tube produced.

7- Development of appressoria.

For measurement of conidia from different collections were stained in cotton blue and mounted in lactophenol. In each case 100 conidia were measured with the help of ocular micrometer. The difference in the size of conidia was statistically analyzed to find out the morphological difference among them, if any. For fibrosin bodies and types of germ tubes produced, fibrosin bodies test and germination test were performed.

2.1. Fibrosin body test:

Conidia from each sample were tested for the presence of fibrosin bodies. These were gently dusted on clean dry glass slides from different samples. Later on a few drops of 3% KOH (Potassium hydroxide) aqueous solution was added on each slide (Kable and Ballantyne 1963). While, examining the conidia under the microscope, following observations were taken in to consideration:

1- Presence or absence of fibrosin bodies.

2- Percent occurrence of conidia with fibrosin bodies.

3- Number of fibrosin bodies per conidium.

2.2. Germination test:

Similarly, for testing germination of conidia, it was gently dusted on clean dry glass slide obtained from different leaves or other aerial parts of the plant samples. These slides were placed on triangle glass rod kept in Petriplates containing sterilized distilled water just touches the upper surface of the rod. The Petriplates were incubating at 20^0C for 24 hours. After incubation period these slides were

selected randomly in different microscopic field. Following observation were made for the germ tube characters:

1- Point of origin of germ tube on the conidia terminal/sub-terminal/lateral.

2- Single germ tube simple/fork.

3-Presence or absence of appressoria at the tip of the germ tube.

2.3. Consistency in anamorph characters:

To ascertain consistency in the conidial character in order to use them as basis for identification of *E. cichoracearum* var. *cichoracearum* and *S. fuliginea*, dimension of conidia (length and breadth), presence and absence of fibrosin bodies, percent conidial germination, point of origin of germ tube on conidia, morphology of germ tube, percent forking of germ tube and development of appressoria in various samples of plant infected with powdery mildew were studied.

3. Intensity of disease:

To determine the intensity of disease on qualitative basis, based on visual observations, about 50 to 100 infected plants of each species of weed were selected and intensity of disease was categorized as suggested by Khan *et al.* (1974) as follows:

1-No infection (-) = No visible disease symptoms

2-Mild infection (+) = Powdery patches few, small in size and scattered

3-Moderate infection (++) = Patches many and large in size

4-Severe infection (+++) = Large patches covering almost the entire leaf area

The intensity of the disease on different weeds in and around Aligarh was also compared. The inoculum from different localities was maintained on young seedlings of respective weeds grown in pots containing autoclaved soil in the glass

house. After tentative identification, different species of powdery mildews was maintained separately. Artificial inoculation of plants in glass house was done by dry dusting of conidia from infected leaves on leaf surfaces of the healthy plants of each weed.

4. Frequency of powdery mildew disease (%):

The percentage of frequency of occurrence of powdery mildew disease was calculated by following formula:

$$\text{Frequency of powdery mildew disease (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

5. Pathogenicity test:

To determine, the infection of *E. cichoracearum* var. *cichoracearum* on weeds viz., *Parthenium hysterophorus, Acalypha indica, Ageratum conyzoides, Cissampelos pareira, Coccinia cardifolia, Euphorbia hirta, Melilotus indica, Solanum nigrum* and *Vernonia cineria* 3-4 week-old seedlings of these weeds were inoculated with conidia of this powdery mildew fungus by brushing infected leaf materials onto the leaves of tested weeds. The inoculated leaves showed typical powdery mildew symptoms within 5-8 days, which first appeared as white-powdered colonies and subsequently coalesced with abundant growth covering the entire leaf surface. Similarly, to determine the effect of powdery mildew disease caused by *E. cichoracearum* var. *cichoracearum* and *S. fuliginea* on the growth of *P. hysterophorus.* 3-4 week old seedlings of *P. hysterophorus* were inoculated with either *E. cichoracearum* var. *cichoracearum* or *S. fuliginea.* Uninoculated plants served as control. Each treatment was replicated three times.

6. Preparation and sterilization of soil mixture:

Sandy loam soil collected from a fallow field of AMU Aligarh farm was sieved through 16 mesh sieve and mixed with sieved river sand and organic mixture in ratio of 3:1:1, respectively. Earthen pots, 10 inch were filled with this soil mixture at the rate of 4kg/pot. A little amount of water was poured in each pot to just wet the soil before transferring to an autoclave for sterilization at 20 lb pressure for 20 minutes. Sterilized pots were allowed to cool down at room temperature before use for experiments.

7. Raising and maintenance of test plants:

Seeds of *P. hysterophorus*, surface sterilized with 0.1% mercuric chloride for 2 minutes and washed thrice in sterilized water were sown at the rate of 5 seeds/pot. After their germination thinning was done so as to maintain only one plant per pot. Three week old, well established and healthy seedlings were used to study the impact of powdery mildew disease caused by *E. cichoracearum* and *S. fuliginea* on the growth of *P. hysterophorus.*

8. Plant growth determination:

Plant was uprooted after 90 days of inoculation. Roots are washed thoroughly in slow running tap water. Utmost care was taken to avoid loss and injury of root system during the entire operation. For measuring length and weight, the plants were cut with a sharp knife just above the base of root emergence. Length of shoot and root was recorded in centimeters from the cut end to the tip of first leaf and the longest root respectively. The excess water of plants was removed by putting them between the two folds of blotting sheets for some time before weighing them separately. The weight was recorded in grams. For measuring dry weight, the shoots and roots were kept in envelops separately for drying in an oven

running at 80^0C for 24 hours. Besides, the data on number of heads/plant, percentage of viable pollen grains and germination of seeds were also recorded.

9. Statistical analysis:

The data were analyzed by one way analysis of variance (ANOVA) using SPSS 12.00 software (SPSS Inc., Chicago, IL, USA). C.D. was calculated at P=0.05 and at P=0.01 to test for significant differences.