Chapter III
Materials
and
Methods

Satara is the one of the districts of Maharashtra, located within $16^0.50'$ to $18^0.10'$ N latitude and $73^0.45'$ to $75^0.0'$ E longitude. Satara district is spread over 10580 sq.km. area (Gazatter of Satara district). Satara district is located at the cushions of the Sahyadri ranges.

Main hill features of the Satara are Ajinkyatara, Yewteshwer and Kas towards west, Pateshwar toward South, Mahabaleshwar towards North West. The soil varies from tract to tract. Mostly it covers the basaltic to laterite which gives rise to red loamy soil, black cotton and clayey soil rich in humus. Climate of Satara is similar to the climate of the whole Maharashtra through of the year different and distinct seasons are recognized, these are Summer – February to May, Monsoon – June to Sept., Post-Monsoon – October to mid December and Winter – mid December to February.

In the present study investigation of groundnut and gram crops seeds were carried by taking following aspects.

i) Field observation by photographic method

ii) Seed treatment of seeds of the crops groundnut and gram.

iii) Identification of fungal colonies in control blotter plates.

Field observation :

The groundnut crop is generally taken during the Kharip season and gram crop is taken during the Rabbi season.

Field observation for groundnut and gram crop from villages from Satara district. The field observations were made by visiting the fields periodically as per the growth of plants upto seedling stage.

The plants were observed for

1) Germination of seeds.

2) Post germination development

3) Seedling formation

First the fields were selected where groundnut and gram crops were taken. Then from sowing time the fields were observed for the growth of seeds.

- **Seed treatment of seeds of the crops groundnut and gram :**

Seed treatment is probably the cheapest and often the safest method of direct plant disease control.

Seeds are attacked by many seed borne fungi. For the control of such pathogens chemical treatment with various chemicals was useful. For this two chemicals were tried namely sulpher and mercuric chloride.

Sulpher is one of the most important fungicides used for the control of various diseases. Elemental sulpher occurs in both free state and also in combination. Sulpher occurs in many allotropic forms, of which α or the rhombic. Sulpher is insoluble in water, slightly soluble in alcohol and ether and freely soluble in carbon disulphide, sulpher chloride and hot benzene. The specific gravity of the rhombic form is 2.07 and the M.P. is 112.8^0C. Sulpher is being widely used in finely ground condition or in the colloidal form in dust preparation.

Mercuric chloride ($HgCl_2$) is a corrosive sublimate. It is white crystalline substance with a specific gravity of 5.42, M.P. of 265^0C and B.P. 303^0C. It is soluble upto 5.7 to 54 parts in cold and hot water respectively. It decomposes in the presence of hydroxides including sodium, calcium and potassium.

Kellerman and swingle in 1878 tested mercuric chloride ($HgCl_2$) for the first time as a fungicide, for seed treatment on cereals. Hiltner, German worker, successfully used mercuric chloride for the control of fusarium disease on rye in

1910. However, its high toxicity to man, animals and plants made it necessary to seek more active and safer compounds for use in agriculture. Many of the organomercury compounds at the concentration used are not only harmless to plants but even stimulate their growth. Sometime the stimulation is so great that it leads to a substantial increase in yield.

Arachis hypogeae (Groundnut) and *Cicer arietinum* (Gram) seeds are used for seed treatment.

Sulpher Treatment : Groundnut

Groundnut (*Arachis hypogeae* L.) a leguminous oil seed crop, is grown in Kharip and in rabi/summer seasons. For getting good yield and higher oil content of groundnut, treatment is essential.

For the experiment ground variety JL-24 seeds of groundnut were taken at random for sulpher treatment. Seeds were treated with 1%, 2%, 5% concentration and the time period for each concentration were 6, 12 and 24 hours respectively. Treated seeds are after appropriate treatment were thoroughly washed with water to remove the chemical from the surface of seeds. Then seeds were kept for germination and after 24, 48, 72 etc. hours germination percentage was noted down and observation table were prepared.

Also the seeds were checked for appearance of seed borne fungi on them, by keeping untreated petriplates as control.

$HgCl_2$ treatment : *Arachis hypogeae* L. (Groundnut)

Groundnut crop is attacked by many seed borne fungi like mucor, Aspergillus, Rhizopus, Penicillium and Rhizoctonia and others. For the control of such fungi $HgCl_2$ seed treatment is useful.

In the present study various concentration of $HgCl_2$ have been tried by changing period of treatment.

For the experiment 10 seeds of groundnut were taken at random, which is a constant number for all treatments. Seeds were treated with 0.1%, 0.2%, 0.05% concentration and the time periods for each concentration were 6, 12 and 24 hours respectively. The treated seeds are after appropriate treatment were thoroughly washed with water to remove the chemical from the surface of seeds. Then seeds were kept for germination and after 24, 48, 72 etc. hours germination percentage was noted down and observation table were prepared.

Also the seeds were checked for appearance of seed borne fungi on them for this untreated plate was kept as control.

***Cicer arientinum* (Gram) :**

Sulpher treatment :

Gram is one of the most extensive used pulses in India. It is grown in rabi seasons.

For the experiment 10 seeds of gram were taken at random which is constant number for all treatments. Seeds were treated with 1%, 2%, 5% conc. and the time period for each concentration were 6, 12 and 24 hours respectively. Treated seeds are after appropriate treatment were thoroughly washed with water to remove the chemical from the surface of seeds. The seeds were kept for germination in ( ) and after 24, 48, 72 etc. hours germination percentage was noted down and observation tables were prepared.

Also the seeds were checked for appearance of seed borne fungi on them, by keeping untreated petri plate as control.

$HgCl_2$ Treatment :

Gram seeds are is attacked by many seed-borne fungi like, *Aspergillus, Rhizopus, Fusarium* and others. For the control of such fungi seed treatment is useful. In the present study various concentration of $HgCl_2$ have been tried by changing period of treatment.

For the experiment 10 seeds of groundnut were taken at random, which is a constant number for all treatments. Seeds were treated with 0.1%, 0.2%, 0.05% concentration and the time periods for each concentration were 6, 12 and 24 hours respectively. The treated seeds are after appropriate treatment were thoroughly washed with water to remove the chemical from the surface of seeds. Then seeds were kept for germination and after 24, 48, 72 etc. hours germination percentage was noted down and observations were prepared.

Also the seeds were checked for appearance of seed borne fungi on them for this untreated plate was kept as control.

Identification of fungal colonies of control blotter plates :

Seed always harbour diverse groups of microorganisms. Seed borne fungi, in particular becomes very important for the purpose of evaluation of its planting value (viability), seed treatment, plant quarantine, seed certification etc. several methods are available for the detection of microorganism associated with seeds. The selection of methods used for the detection of seed borne pathogens depends upon purpose of the test. 'Blotter method' have been recommended by the ISTA (1966) for routine examination of crop seeds for fungal infection.

To evaluate seed samples for associated seed borne fungi, seed samples of groundnut (*Arachis hypogeae*) and gram (*Cicer arietinum*) crop were collected from farmers, crop fields, local market in and around Satara.

Seeds were collected and representative seed samples obtained were stored in clean plastic containers. These seeds wee used for the study of seed borne fungi and seed treatement.

- **Visual evaluation of seed-borne fungi :**

Visual evaluation were carried out by following method.

i) Examination of dry seeds is a quick method for detections of seed-borne pathogens. The seeds from representative samples were observed by unaided eye to find out the difference in size or colour.

ii) Examination under a binocular :

The untreated seeds were examined under a stereo binocular bright field microscope to make out external symptoms and contaminants present on the surface of seeds.

Blotter method for control :

Doyer (1938) was first who used filter paper for identification of seed borne fungi. The Blotter method is a simple method used for detecting the seed borne fungi associated with seeds. It is used for detecting those fungi which are able to produce mycelia growth and fruiting structures during incubation.

Blotters were sterilized and soaked in sterile distilled water. They were place in sterilized petriplates. Four to six seeds were placed equidistant from one another. After placement of seeds, culture plates were incubation at room temperature (28^0C – 32^0C) for five to ten days. Slides were prepared for necessary observations.

Control plate :

During all these treatment and their observation control plate was kept where seeds were only washed with water. Control plate is called as untreated

blotter plate as it was not treated with chemical. After washing the seeds were placed for germination by lebelling the petri dish as control plate

Identification of seed borne fungi from control plate :

Fungi on seeds appear more readily. Slides were prepared for observation. The fungi were identified up to generic level using the "Illustrated Genera of fungi Imperfection" by Barnett (1973) and identification upto species level was done with the aid of Ellis (1976), Subramanian (1961), Tandon (1968), and Kamat (1959) Thom and Raper key for *Aspergillus* were used from Subramaniam (1961) etc.