Chapter V

Summar nclusions

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 10.0962 sq. km. area (Gazzatter of Satara, 1991.). Satara district is located at the cushions of the Sahyadri ranges.

Main hill features of the Satara are Ajinkyatara, Yewteshwer and Kas towards west, Pateshwer towards 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 though of the year different and distinct seasons are recognized, these are Summer- February to May, Monsoon- June to Sept, Post-Monsoon – October to mid. September and winter –mid December to February.

Main source of precipitation is the South-West Monsoon; annual average rainfall recorded during study was 1571.1 mm in 2006 and about 1274.7 mm in the year 2007. Temperature ranges from about 0 to 1^0C at Mahabaleshwar as lowest temperature of Satara district, while highest average temperature remains at about 39^0C toward eastern side of the district. The maximum and minimum temperature of Satara (Town) recorded during study, were, 37.9^0C (2006), 38.2^0C (2007) and 12.5^0C (2006), 13.6^0C (2007) respectively.

Average humidity varies from 98% to 42%. Maximum average humidity recorded during study was 98% (2006, 2007) while minimum average humidity recorded was 42% (2006) and 22% (2007). Monsoon humidity values are maximum, while humidity remains very low during summer months. (Meteorological Department of India, Satara). Warm and humid climate in the western region of the Satara favors luxuriant growth of vegetation therefore western region of Satara i. e. Koynanager, Kas, Mahabaleshwar is rich in vegetation and shows angiosperm diversity.

Favorable climatic condition and associated rainfall in most part of the Satara district is excellent for agriculture. Agriculturally Satara is advanced and most of the cereals like rice, wheat, jowar, bajara, pluses like pigeon pea, gram, black gram, pea etc. vegetables like bringal, potato, bens, okra and oil seeds like groundnut, soybean, sunflower etc. are successfully cultivated in the district. The warm and humid conditions are also favourable for growth of saprophytic fungi and bacteria. Favourable climatic conditions and availability of host as well as substratum are responsible for the growth of the fungi in this region. The warm and humid condition during monsoon favours the growth of microorganisms like fungi, cause deterioration of seeds during this season of the summer.

Considering this relation among fungal pathogens, available hosts and climatic conditions, a study, on the investigation of seed mycoflora associated with cereals, pulses, oil seeds, and vegetable seeds, was undertaken.

Cereals considered to study the mycoflora associated with seeds were Sorghum, Wheat, Maize; pulses taken for study were Pigeon pea (Tur), Gram, Pea and Urdbean while Oil seeds studied were Groundnut, Soybean and Sunflower. Vegetables included in this study to explore seed mycoflora were, Okra, Chilli, Cluster bean and French bean.

Seed samples were collected from local farmers and used for the present investigation. The study of mycoflora associated with the seeds includes physical analysis of seeds and study of ectophytic and endophytic mycoflora of seeds.

Visual and physical analysis of the crop seeds considered for study, seeds carried to find out amount of inert materials has shown that, the maximum percentage of inert material was present (9%) in Sorghum (*Sorghum bicolor* L. Moench) while minimum inert material observed in the seeds of French bean (*Phaseolus vulgaris* L.) which was about 2%. The visual analysis also revealed that the maximum percentage of 13 of discoloured and disfigured seeds was present in sorghum (*Sorghum bicolor*

L. Moench) and groundnut (*Arachis hypogaea* L.) while minimum percentage of discoloured and disfigured seeds 3% was recorded in the chilli (*Capsicum annum* L.) seeds.

During the present investigation, ectophytic as well as endophytic mycoflora mycoflora associated with the seeds of the crop were considered for the present study was revealed.

Ectophytic mycoflora were studied by using Blotter Method and Agar Plate Method. Seed Suspension and Dry Seed Inoculation were used in the agar plate method in which the seeds were incubated on CZ medium. In the blotter method the dry seeds were incubated on wet blotting paper. In both the methods the petriplates with seeds were incubated at room temperature and observations were made regularly.

To reveal endophytic mycoflora seeds were surface sterilized in 1: 1000 mercuric chloride solutions, washed several times with distilled water and were split open. These split opened seeds were placed on the agar and were incubated at room temperature. The incubated seeds were regularly observed; fungi developed were recorded and identified after preparation of slides. The results of the seed incubation and fungi developed are discussed below.

Cereals considered to study the ectophytic and endophytic mycoflora were Sorghum, Wheat and Maize.

The methods used for studying ectophytic mycoflora of ***Sorghum bicolor* L. Moench.** (Sorghum) have revealed 31 species of the fungi associated with seeds. Among these *Aspergillus* with 17 species and *Alternaria* with 6 species were observed as dominant forms. In addition *Penicillium* with 3 species and *Rhizopus* with 2 species, were observed as subdominant form. Rest of the fungal species associated with Sorghum seeds were represented by *Mucor, Actinomucor, Verticillium* each with single species. Forms found specific to sorghum seeds during present investigation, were *Alternaria brassicae, A. carthami, A. citri, A.*

humicola, Mucor javanicus and *Penicillium corylophilum.* Better results were observed with Blotter as compared to Agar Plate Method.

Eleven species of ectophytic seed mycoflora of ***Triticum aestivum* L.** (Wheat) belonging to five genera were detected from seeds by using Dry Seed Inoculation Test, Seed Suspension Method and Blotter Method. 6 species of *Aspergillus* and 2 species of *Penicillium* were isolated along with *Rhizopus artocarepi, Alternaria alternata, Curvularia barreriae. Curvularia barreriae* and *Penicillium nigricans* were found specific to the seeds of Wheat. In the present study, Seed Suspension Method showed better result than Blotter Method and Dry Seed Inoculation Method. While the methods used to explore the endophytic mycoflora have revealed four species belonging to three genera, i.e. *Aspergillus amstelodami, A. fonsecaeus, Cladosporium cladospoiroides, Rhizopus combodia.* In present examination, of ectophytic mycoflora, total number of 32 species of fungi belonging to 5 genera were isolated from the seeds of ***Zea mays* L.** (Maize) by using three methods mentioned earlier. *Aspergillus* was dominant genus displaying 25 species followed by *Penicillium* with 4 species. Rest of the fungal species associated with maize seeds were represented by single species of each of *Alternaria, Rhizopus* and *Verticillium.* Two species of *Penicillium; P. atramentosum* and *P. rugulosum* were specific to seeds of maize. Among these techniques Blotter Method proved to be better than Seed Suspension Method and Dry Seed Inoculation Method. While the methods used to explore the endophytic mycoflora have revealed four species belonging to two genera, which were *Aspergillus chevalieri, A. niger, A. pulverulentus* and *Cladosporium cladosporioides.*

Fungal species *Alternaria burnsii* was detected during the present investigation with two cereals, Sorghum and Maize and *Penicillium citrinum* was isolated from Wheat and Sorghum.

Pulses considered to study of ectophytic and endophytic mycoflora were Pigeon pea, Gram, Pea and Urdbean.

The methods used for studying ectophytic mycoflora of ***Cajanus cajan* (L.) Millsp.** revealed about 20 fungal species belonging to 2 genera. *Aspergillus* was dominant genus with 19 species and *Penicillium* with single species. *Penicillium oxalicum* was observed on seeds of pigeon pea. In the present analysis, Dry Seed Inoculation Method gave better results than Blotter Method and Seed Suspension Test. While the studies carried out to find out endophytic mycoflora revealed four fungal species, *Aspergillus amstelodami, A. chevalieri, A. niger* and *A. oryzae*.

From the seeds of ***Cicer arietinum* L.** (Gram) 23 species of fungi were observed during the study of ectophytic mycoflora. They belong to 10 genera. Among these *Aspergillus* with 9 species was observed as dominant forms, in addition *Alternaria* with 3 species and *Rhizopus* with 2 species, were observed as subdominant form, rest of the fungal genera associated with chickpea seeds were represented by were *Cladosporium, Curvularia, Drechslera, Mucor, Penicillium, Verticillium* each with single species. Fungal forms found specific to the seeds of Gram were *Alternaria dianthicola, A. tenuis, A. tenuissima, Drechslera australiensis, Fusarium moniliformie, F. solani, Penicillium purpurgenum, Rhizopus nodosus*. In the present investigation, Seed Suspension Test proved to be best for study of seed mycoflora than Dry Seed Inoculation Method and Blotter Method. While the methods used to explore the endophytic mycoflora have been revealed four species belonging to two genera, which were *Aspergillus amstelodami, A. chevalieri, A. niger* and *Rhizopus combodina* on seeds of Gram.

From the seed of ***Pisum sativum* L.** (Pea), 16 fungal species belonging to 6 genera were observed during the study of ectophytic mycoflora. *Aspergillus* was dominant with 11 species and 5 genera represented by single species detected were *Cladosporium, Curvularia, Penicillium, Mucor* and *Rhizopus*. In the present examination Dry Seed Inoculation Method proved to be best for study than Seed Suspension Test

and Blotter Method. However the methods used to explore endophytic mycoflora of pea couldn't result.

The methods used for studying ectophytic mycoflora of ***Vigna mungo* (L.) Hepper.** (Urdbean) have revealed about 16 fungal species belonging to 5 genera associated with seeds. The genus *Aspergillus* was dominant and the genera *Fusarium, Penicillium, Mucor* and *Rhizopus* each with single species were detected from seeds of urdbean. Fungal forms found specific to seeds were *Aspergillus awamori, A. phaeocephalus* and *Penicillium janthinellum.* In the present study Seed Suspension Test proved to be best than Dry Seed Inoculation Method and Blotter Method. However, the methods used to explore endophytic mycoflora of urdbean couldn't result.

Oil seeds considered to study ectophytic and endophytic mycoflora are Groundnut, Soybean and Sunflower.

While studying ectophytic mycoflora of ***Arachis hypogaea* L.** (Groundnut) about 43 species of fungi belonging to 6 genera were revealed with seeds. Genus *Aspergillus* ranked first displaying 33 species followed by *Penicillium* (3), *Rhizopus* (3), *Mucor* (2), *Alternaria* (1) and *Fusarium* (1). Fungal species confined only to seeds of groundnut were *Aspergillus atropurpureus, A. luchuensis, A. nanus, A. sclerotiorum, Mucor circinelloides* and *M. griseo–cyaneus.* In the present analysis, Blotter Method gave better result than with Dry Seed Inoculation Method and Seed Suspension Test. The studies carried to find out endophytic mycoflora have, revealed five fungal species belonging to two genera. Fungal species *Aspergillus amstelodami, A. chevalieri, A. candidus, A. niger* and *Rhizopus combodia* were reported.

The methods used for studying ectophytic mycoflora of ***Glycin max* L. Merril.** (Soybean) revealed about 20 species belonging to 8 genera were obtained by three methods, Blotter Method, Dry Seed Inoculation Method and Seed Suspension Test. The genus *Aspergillus* with 13 species was dominant. *Alternaria, Cladosporium, Curvularia, Dictylaria, Penicillium,*

Fusarium and *Rhizopus* represented each with single species. In the present examination, Blotter Method has given better results; it may be because the moisture provided for fungal growth. While considering, endophytic mycoflora, four fungal species belonging to two genera were recorded. Which were *Aspergillus fonsecaeus, A. fresenii, A. niger* and *Cladosporium cladosporioides.*

From the seeds of ***Helianthus annus*** **L.** (Sunflower) during the study of ectophytic mycoflora only three fungal species, developed by using three methods, were *Aspergillus niger*, *Aspergillus insecticola* and *Actinomucor*, while during this study endophytic mycoflora were not observed.

Vegetable seeds considered to study ectophytic and endophytic mycoflora were Okra, Cluster bean, Chilli and French bean.

Taking into account of ectophytic mycoflora, 8 species belonging to 5 genera were isolated from seeds of ***Abelmoschus esculentus*** **L.** (Okra). Amongst these *Aspergillus* with 4 species was dominant. The genera *Cladosporium, Fusarium, Mucor* and *Rhizopus* were representing by single species. While the methods used to explore the endophytic mycoflora have revealed, five fungal species belonging to three genera, which were *Alternaria palandui, Aspergillus amstelodami, A. niger, A. quercinus* and *Cladosporium cladosporioides.*

In view of total number of ectophytic mycoflora of ***Capsicum annum*** **L.** (Chilli) 9 fungal species belonging to 6 genera were observed. The genus *Aspergillus* with 4 species was dominant. The genera *Actinomucor*, *Cladosporium, Fusarium, Rhizopus* and *Trichocladium* were each with single species were detected from seeds of chilli. In the present investigation Seed Suspension Test was found to best for study. During this study, endophytic mycoflora were not recorded.

Ten fungal species belonging to three genera were reported from seeds of ***Cyamopsis tetragonoloba*** **Taub.** (Cluster bean) during the study of ectophytic mycoflora. *Aspergillus* was dominant genera representing 8

species. *Cladosporium* and *Rhizopus* were represented by one species each. In the present analysis, Blotter Method gave better result than Dry Seed Inoculation Method and Seed Suspension Method. While the methods used to explore the endophytic mycoflora have revealed, three fungal species belonging to two genera were recorded. Fungal species *Alternaria palandui*, *Aspergillus amstelodami* and *A. niger* were reported.

The methods used for studying ectophytic mycoflora of ***Phaseolus vulgaris*** **L.** (French bean) have revealed about 28 fungal species belonging to 5 genera with three methods, Blotter Method, Dry Seed Inoculation Method and Seed Suspension Test. The fungal species, *Aspergillus* was dominant genera representing 23 species. Three genera, *Curvularia, Dictyoarthrinum* and *Torula* were represented by single species and *Penicillium* was represented by two species. While the methods used to explore the endophytic mycoflora have revealed three fungal species which were *Aspergillus niger, Cladosporium cladosporioides* and *Rhizopus combodina.*

Some specific results about the seed mycoflora showed that; *Actinomucor* was detected from the seeds of Sorghum, Sunflower and Chilli. *Alternaria alternata* was reported from Wheat, Soybean and Groundnut. *Aspergillus alutaceus* was isolated from seeds of the Maize, Groundnut and Cluster bean. *A. castaneus* was recorded from Maize and Cluster bean. *A. nidulance* was detected from Soybean, Pea, Groundnut and French bean. *Cladosporium cladosporioides* was reported from the seeds of Wheat, Maize, Soybean, Gram, Pea, Okra, Freach bean, Chilli and Cluster bean. *Curvularia lunata* was Gram, Soybean, Pea and Freach bean. *Verticillium* was detected from seeds of Sorghum, Maize and Gram.

Species of *Mucor, Rhizopus* appeared earlier during culture followed by *Aspergillus, Alternaria Penicillium, Fusarium* and *Verticillium* appeared later. Ectophytic mycoflora were studied by using Blotter Method and Agar Plate Method. Seed Suspension and Dry Seed Inoculation were used in the agar plate method; the seeds were incubated on CZ medium. In the blotter

method the dry seeds were incubated on wet blotting paper. In both the methods the petriplates with seeds were incubated at room temperature and observations were made regularly.

To reveal endophytic mycoflora seeds were surface sterilized in 1: 1000 mercuric chloride solutions, washed several times with distilled water and were split open. These split opened seeds were placed on the agar and were incubated at room temperature. The incubated seeds were regularly observed; fungi developed were recorded and identified after preparation of slides. The results of the seed incubation and fungi developed are discussed below.

The suggestion to the farmers:-

Losses due to deterioration of grains by storage fungi can be reduced by the following methods

1) Proper precaution at the harvest and during post harvesting operations helps to reduce occurrence of storage fungi. Seeds which are clean, undamaged and dried are ideal for storage.
2) Drying the grain to below 14 percent moisture and storage on cool with limited oxygen are important steps for a fairly safe storage of grains. Sanitary measures such as reduction in dust and fines before storage also help.
3) Solar heat treatment is best method to reduce moisture from seeds.
4) Hot water treatment has been standard method for controlling embryo infection.
5) Seed treatment should be primarily used to provide additional security that disease free seeds has been obtained
6) Crop rotation is the cultural practice to control seed borne infection.
7) Seeds are stored at the low temperature to reduce the infection i.e. cold storage method is used. Use the proper storage bins.
8) Use the certified seeds or disease resistant variety for cultivation in the every year.