SECTION - I

GENERAL
GENERAL INTRODUCTION

'Foot-rot' is an important disease of wheat in India. It occurs in almost all the wheat growing regions specially in the central parts of the country and Maharashtra and Gujrat. The losses are difficult to assess but it is estimated to cause damage amounting to 0.8% to 14.7% (Asthana, 1954-55) of the total yield which is quite considerable for such an important crop. Sagar, which is situated in Madhya Pradesh, is an important centre of wheat production where this disease is a special problem confronting the wheat growers.

The disease which is termed as 'foot-rot' shows a multiple syndrome caused by a variety of micro-organisms. As it is widespread in its distribution all over the wheat growing regions of the world, it has attracted the attention of plant pathologists of all these countries. The most notable workers in this field are Garrett who worked in Australia and Simmonds who has been working in Canada. In India also attempts have been made by several workers to study the etiology of this disease, but the work has been rather sporadic in nature and no body has tried to study the disease in the light of recent findings on the ecology of soil micro-organisms as they affect the pathogenicity and survival of the soil-borne diseases. As is now known, the pure culture pathogenicity tests which have been the routine for such studies, give us a very distorted picture of the actual phenomena associated with these diseases.
The elimination of the soil organisms from the site of micro-environment involving the disease smuts out an important factor in the entire picture of interactions which could not be appreciated by some of the earlier workers of this field. In India particularly, this aspect of the study was never kept in view and on that account many erroneous conclusions were naturally drawn.

It is in the above light that the present investigations were undertaken with a view of working out the details of this important disease. These studies fall under the following headings:

I. **Etiological studies** - In this, besides close observation in the fields, the isolation of the organisms causing the disease and tests for their pathogenicity under as natural conditions as possible were made.

II. **Rhizosphere studies** - The rhizosphere and rhizoplane microflora of the normal healthy plant and its succession were studied and it was compared with the modified flora of the attacked plants. Further, a comparative study of the rhizosphere flora of two varieties of wheat different in their susceptibilities was also made.

III. **Biological interaction** - The interactions between the pathogens and the possible soil antagonists against them were studied both in agar plates and soil.

IV. **Saprophytic behaviour** - The competitive saprophytic ability and saprophytic survival of the different pathogens were studied.
Brief Resume of Earlier Work

The foot-rot or root-rot maladies of cereals are well known to the farmers of all wheat growing countries. The disease was known as 'take-all' by the farmers of Australia since long, although the causal organism was declared correctly in 1890 by Prilleux and Delacroix from France. The disease appears in the form of a syndrome caused by a variety of organisms. The symptoms have been variously described, the differences being due to the attack by various organisms singly or in combinations. On account of these differences a number of names have appeared in the literature such as 'pre-emergence blight', 'seedling blight', 'take-all', 'white heads', 'deaf ears', etc. (Samuel, 1928; Samuel and Garrett, 1932; Bennett, 1928; Garrett, 1936; Oswald, 1950; Hyne, 1935). The organisms reported by various workers are: Ophiobolus graminis, Bipolaris sorokiniana (=Helminthosporium sativum) and other species, Gibberella saubinetii, Fusarium culmorum and other spp., Rhizoctonia solani, Pythium spp., Sclerotium rolfsii and Curvularia spp. The literature on this subject has been very well reviewed by Stevens (1919), McKinney (1925), Bockmann (1936), Garrett (1944, 1956) and Simmonds (1939, 1941, 1953).
Work in India: In India the work on foot-rot disease has been done mainly by the Government plant pathologists. As early as in 1917 Subramaniam reported a disease of wheat from Mandalay (Burma) and later in 1920 from Central Provinces (included in present Madhya Pradesh). The causal organism was identified as *Rhizoctonia destruens*. A little later (1928) the same author described *Pythium graminicolum* causing root-rot. This was followed by McRae (1922, 1923, and 1924) who stressed on the huge losses caused by the disease and isolated several organisms: *Helminthosporium* spp., *Acreiothecium* sp., *Alternaria* sp., and *Rhizoctonia* sp. Dastur (1928, 1935, 1937) reported two species of *Helminthosporium* and four other fungi, *Acreiothecium* sp., *Fusarium* sp., *Phoma* sp., and *Ophiobolus* sp. as isolated from black point disease of wheat grains from Sindewahi (M.P.). He later noted that some of these organisms were responsible for foot-rot also. Mitra (1931) studied different isolates belonging to 7 species of *Helminthosporium* which were isolated from cultivated grasses (one from ginger), on wheat and some other hosts. In 1940 Padwick reported 'take-all' disease of wheat for the first time from India and isolated *Q. graminis* from the diseased parts. He, however, failed to get perithecia either from nature or in culture. He also carried out pathogenicity experiments (1942) with his isolate of *Q. graminis* and *Fusarium avancense*. 
A scheme on foot-rot disease of wheat was taken up for the Central Provinces and Berar region (1954-58) by the Department of Agriculture. They made a survey of the disease and isolated four fungi, viz. Helminthosporium rostratum (=H. rostrata), Sclerotium rolfsii, Curvularia lunata and F. moniliforme. Much of this work was confined to finding the effects of agricultural practices on the disease such as time of sowing, manurings, soil types, etc. Apart from these, reports are available of some sporadic works of minor importance. Chattopadhyay (1953) found S. specifera and S. rolfsii as cause of foot-rot and root-rot disease respectively from West Bengal. He also did some pathogenicity experiments with these fungi.

Further details of the above quoted works are given at the appropriate places in the text of this thesis when different aspects of study are dealt with.
CLIMATE OF SAGAR AND PREVAILING AGRICULTURAL
PRACTICES IN THE CULTIVATION OF WHEAT

Sagar is situated in north of the Madhya Pradesh state at the latitude of 23.50 N, longitude of 78.50 E and an altitude of 2,000 feet. The climate is typically monsoonic. The average annual rainfall is 48 inches, the bulk of which falls during the rainy season from June to September. There is little rain during winter months, most of which falls in December and January. Average rainfall on monthly basis for the last ten years is given in Table No. 1. The general averages for mean maximum and minimum and the highest maximum, minimum temperature for the twelve months for five years, 1955-59 are also given in Table No. 1. It shows that the maximum temperature is reached in May and then it gradually falls reaching the minimum in January after which it again starts rising up to May.

Soil temperature: Data of soil temperature of Sagar University Campus of the superficial layers (5 cm.) shows the following points (Table No. 1). The minimum of the year is found to be 16°C in January, while the highest (46.5°C) is reached in May. The surface portion gets still more heated and the temperature may reach up to 57°C in May. During the growing season of the crop, i.e., from October to March, it
Table No. I.
Climatic data of Sagar.

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature in °C. Average for 5 years (1955-59)</th>
<th>Rainfall in mm. Average for 10 years (1951-1959)</th>
<th>Soil temperature at the depth of 5 cm. °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Max.</td>
<td>Mean Min.</td>
<td>Highest Max.</td>
</tr>
<tr>
<td>January</td>
<td>18.62</td>
<td>24.7</td>
<td>12.34</td>
</tr>
<tr>
<td>February</td>
<td>20.71</td>
<td>27.16</td>
<td>14.26</td>
</tr>
<tr>
<td>March</td>
<td>25.75</td>
<td>32.44</td>
<td>19.06</td>
</tr>
<tr>
<td>April</td>
<td>30.73</td>
<td>37.28</td>
<td>24.18</td>
</tr>
<tr>
<td>May</td>
<td>32.92</td>
<td>39.18</td>
<td>26.66</td>
</tr>
<tr>
<td>June</td>
<td>31.26</td>
<td>39.18</td>
<td>25.34</td>
</tr>
<tr>
<td>July</td>
<td>26.07</td>
<td>29.66</td>
<td>22.48</td>
</tr>
<tr>
<td>August</td>
<td>25.62</td>
<td>28.66</td>
<td>22.58</td>
</tr>
<tr>
<td>September</td>
<td>25.36</td>
<td>29.0</td>
<td>21.72</td>
</tr>
<tr>
<td>October</td>
<td>24.47</td>
<td>29.32</td>
<td>19.72</td>
</tr>
<tr>
<td>November</td>
<td>21.38</td>
<td>27.4</td>
<td>15.37</td>
</tr>
<tr>
<td>December</td>
<td>19.4</td>
<td>25.72</td>
<td>13.08</td>
</tr>
</tbody>
</table>
ranges from minimum 16°C to 28°C in January to maximum 28°C to 34.4°C in March.

**Seasons:** The year is clearly divided into three seasons viz., rainy, winter and summer. The rainy season starts by the middle of June and ends by the end of September. The rainy season is followed by winter which lasts up to the end of February. The rainfall is scanty during winter and the period is generally dry. Frosts may occur during this period, specially in the valleys. The summer season commences with March and lasts till the onset of rains. It is marked by hot north-westernly winds and a blazing sun which parches the land. The hottest month is May.

**Soils:** Broadly speaking there are two main types of soils occurring in Sagar formed from the Deccan trap. One which is of red colour is usually found on the plateau and upper parts of the slopes. The second is of black colour and occupies the valleys and lower parts of the slopes. The latter type is commonly known as the "black cotton soils" or "Regur". It is this type of soil which is under cultivation of wheat in valleys. It occurs in various shades, and is further changed due to extensive cropping, manuring etc. It is poorer in drainage and aeration and is utilised occasionally for paddy cultivation after preparation of bunds around the field.

**Cultivation of wheat:** The crop is a winter or "Rabi" crop. Sowing of wheat starts with October, usually in later half and lasts up to the second week of November. For the
wheat cultivation, land is generally kept clean and clear of vegetation. This is particularly the case with dry cultivation which is much prevalent here. By this method moisture is retained for the following crop. The land may also be subjected to cultivation of vegetables or any "Kharif" crop, which can be harvested soon. The kharif crops usually taken up before wheat are Sorghum and paddy. This is the case where irrigation is possible. Irrigation, where possible, is done with well water. Wheat is taken up after Sorghum usually if the latter is spoiled due to some reason or the other. Before sowing proper, soil is prepared with the help of 'Bakkhar' which stirs and loosens the upper 10-15 cm. layer of soil. This is done periodically twice or thrice. Sowing of seeds is done with 'Nari plough' which is a hollow bamboo stick used as a channel for sowing seeds. The seedling level is about 6-10 cm. deep. Before and after working of nari plough the land is levelled with bakkhar.

Average seed sown in the local areas is from 50-100 lb. per acre. In agricultural farms the seed rate is about 60 lb. per acre. Distance between two rows is kept at 25 cm.

Winter rains, if they are in time, are of much help to the growth of the crop. Ears come out in January and February and in March the hot wind starts drying them up and the crop ripens rapidly. It is harvested usually in the later half of March or in April. A part of the produce of the year after threshing etc., is kept as seed for the next
year. Continuous cropping of wheat after wheat is often adopted in many fields.

The local varieties which are most widely under cultivation in local areas are 'Hansia' and 'Pisia'. Both of them tiller well and are high yielding, awned varieties, but are quite susceptible to such diseases as 'foot-rot' and rust. Improved varieties in vogue in the area are Hy 65 and Hy 11.

The crop is cultivated singly or sometimes mixed with other crops. In mixed cropping the counterparts are Cicer arietinum L. (Chana), Lathyrus sativus L. (Teora), Lens esculenta Moen. (Masur), Linum usitatissimum L. (Alsi), Pisum sativum L. (Mattar).

Common weeds growing in wheat fields are Ammophila tenuifolia Cavan., Cocculus hirsutus Diels., Convolvulus arvensis L., Euphorbia geniculata Orteg., Leunaea asplenifolia and Fr.f., Vicia hirsuta Gray, Vicia sativa L. Weeding is often attended but is usually difficult.
MATERIAL AND METHODS

Survey of the fields: Wheat fields are quite abundant in the suburban area of the University site. In view of this intensive survey was mostly confined to the surrounding areas only. Fields were regularly visited fortnightly after sowing and the incidence, type and nature of the disease was noted. The spots with disease were particularly marked for the next season, whenever the crop was repeated.

Collection of diseased material: The plants from the affected patches, diseased as well as apparently healthy, were carefully taken out. Often the affected plants came out very easily breaking up at the lesions or rotten crowns. In such cases the underground system was carefully excavated and brought to laboratory for examination. The collected material was properly dried and pressed to preserve it for future use or reference, with field notes about locality, and date etc.

Microscopic observation of infected portions: The material collected was first examined for external symptoms with the help of binocular microscope. Coloration, type of lesions and external mycelium if any, were the main objects of observations. In some cases the pieces were moistened and sections were cut to observe internal infection. The
non-sporulating hyaline forms could not be easily detected and identified but the method could be used for fungi like Bipolaris spp. with dark hyphae. However, mycelial extension and damage to the internal tissues could be noted by this method. The method was also utilized for noting the host parasite relationship specially in sterile conditions.

**Isolation of pathogenesis:** Advancing margins of infection were selected for isolation. The pieces were either washed thoroughly first by tap water and then by sterilized water and embedded in agar or were surface sterilized. The surface sterilizing agent used at first generally was 0.1% mercuric chloride in 10% alcohol for 50 seconds. The pieces were then repeatedly washed and plated on agar to observe fungal growth. In some particular cases, i.e., black roots and black lesions silver nitrate (1%) was used. Infected pieces were treated for 1 minute with silver nitrate solution, transferred to 1% sodium chloride solution for 1 minute to remove silver nitrate and then successive washings of sterilized water were given to remove excessive salt. The pieces were then embedded in agar. This gave good results in isolation of Onbiobolus graminis which was otherwise very difficult to isolate. The agar medium used for this isolation was mainly potato dextrose agar. The dishes were incubated at 26°C. Growth was generally evident on third or fourth day, but O. graminis took about 4-8 days to appear in agar.
Purification: The mycelial pieces in case of non-sporulating fungi and a needle touch to sporulating portion was taken as inoculum for further purification and this was then inoculated on a fresh dish with a zigzag stroke. Further purification was done as hyphal tip isolation for non-sporulating fast growing members and dilution plates for sporulating forms.

Maintenance of cultures: Cultures thus obtained along with those obtained from rhizosphere and soil plateings were maintained on potato dextrose agar. Transfers were done regularly to maintain the cultures in viable conditions. Intermittently the pathogenic forms were transferred to sterilized wheat straws to restore their sporulating capacity and to retain the wild conditions of Fusarium spp. For other cultural works usual mycological techniques were used.

Identification: The forms were kept under close observations for any variations. Detailed morphological and cultural characters were noted on Czapek's agar, and potato dextrose agar along with micromeasurements. In some cases malt extract agar, Waksman's glucose agar or nutrient agar was used. Camera lucida drawings were made for interesting and important forms. Identifications were done as far as possible with the help of manuals and monographs as by Gilman (1957), Thom and Raper (1945), Raper and Thom (1949), Barnett (1960), Clements and Shear (1931), Wollenweber and Reinking (1935).
Collection of seeds: Wheat seeds of different varieties were obtained from Government Seed Farm, Sagar, and Dr. M.R. Siddiqui, Plant Pathologist, Amravati. Varieties Hy 281, Hy 277, Hy 65 and Hy 11 which are the improved varieties from Powarkheda and are released for circulation were obtained from Government Seed Farm, Sagar. The varieties Vijaya, Kenfad, Mifad 46 and Mifad 345 were obtained from Dr. Siddiqui. Local variety 'Hansia' was obtained locally from the farmers.

Collection of different cultures: Cultures of Bipolaris rostrata (B. rostratum) and Fusarium moniliforme were obtained from Dr. Asthana, Plant Pathologist to Madhya Pradesh Government, Nagpur. The culture of B. sorokiniana was obtained from Indian Type Culture Collection, New Delhi.

Media: The following media were used for various purposes, the composition of which are given below.

i) Czapek (Dox) agar

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium nitrate (NaNO₃)</td>
<td>2.0 gm.</td>
</tr>
<tr>
<td>Potassium phosphate (K₂HPO₄)</td>
<td>1.0 gm.</td>
</tr>
<tr>
<td>Potassium chloride (KCl)</td>
<td>0.5 gm.</td>
</tr>
<tr>
<td>Magnesium sulphate (MgSO₄·7H₂O)</td>
<td>0.5 gm.</td>
</tr>
<tr>
<td>Ferrous sulphate (FeSO₄)</td>
<td>0.01 gm.</td>
</tr>
<tr>
<td>Sucrose</td>
<td>30.0 gm.</td>
</tr>
<tr>
<td>Agar agar</td>
<td>20.0 gm.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml.</td>
</tr>
</tbody>
</table>

ii) Czapek (Dox) + Yeast agar

Above formula with 2.0 gm. yeast powder + 0.2 gm. of streptomycin sulphate.

iii) Plain agar

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar agar</td>
<td>15.0 gm.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml.</td>
</tr>
</tbody>
</table>
iv) Plain agar and straws

1 gm. of chopped straw per tube and addition of plain agar to gel and to prepare slant.

v) Potato dextrose agar

- Peeled potatoes: 200.0 gm.
- Dextrose: 20.0 gm.
- Agar agar: 15.0 gm.
- Distilled water: 1000 ml.

vi) Malt agar

- Malt extract: 25.0 gm.
- Peptone: 1.0 gm.
- Dextrose: 20.0 gm.
- Agar agar: 20.0 gm.
- Distilled water: 1000 ml.

vii) Nutrient agar

- Beef extract: 3.0 gm.
- Peptone: 10.0 gm.
- Agar agar: 15.0 gm.
- Distilled water: 1000 ml.

viii) Waksman's glucose peptone agar

- Glucose: 10 gm.
- Peptone: 5.0 gm.
- Potassium dihydrogen phosphate (KH₂PO₄): 1.0 gm.
- Magnesium sulphate (MgSO₄·7H₂O): 0.5 gm.
- Agar agar: 20.0 gm.
- Water: 1000 ml.

Instead of acidifying the medium, 0.2 gm. streptomycin base was added per litre of medium to check the growth of bacteria.

**Sterilization:** Acidified media were steam sterilized for 10 minutes at 10 lb. pressure, while unacidified media were sterilized at 15 lb. pressure for 15 minutes.
Soil analyses: Two types of soils were used in the experimental work. One was from a wheat field in which wheat was grown year after year without a change in cropping. This has been designated as soil A. The other was from a field in which wheat was not cultivated for the last 4-5 years. This is referred as soil B.

The data on analyses is given in appendix - A.

Besides the methods described above, special methods were used for such studies as pathogenicity, rhizosphere, biological interactions, saprophytic colonization and inoculum potential. These methods are described at appropriate places in the sections dealing with these topics.