CHAPTER X

STUDIES ON PHYLLOSPIRE MICROFLORA OF KUFRI-JYOTI AND UP-TO-DATE CULTIVARS OF POTATO.

The aerial surface of higher plants growing under natural conditions are usually covered with large and varied population of microorganisms. Studies on microbial components of air over crop fields are useful in understanding the aerial phase of ecology of microorganisms, their mode of dissemination in air. This helps in forecasting systems for disease control.

The occurrence of many fungi on aerial surfaces of plants may be related to the inoculum production in the atmosphere which were brought to the leaf surfaces by many environmental agents. Once the microbes settle on the surface, a series of complex events follow due to interaction between surface exudates and the organisms. Interaction also occurs between the pathogens and non-pathogens and also amongst individuals of the same group for growth and survival. The establishment of a plant pathogen on the host tissue is influenced by a large number of factors. Firstly, the physical and chemical constituents of the host plant always try to resist attack by pathogens. The production of phytoalexins and a large number of phenolic compounds and enzymes appears to be a widespread mechanism by which plants attempt to defend themselves against microbes. The anatomy of the host tissue also plays an important role in resistance. Physical factors such as temperature, relative humidity,
light and air currents may also influence the growth and establishment of the pathogen, besides the antagonistic effect of other microorganisms on the persistence of the pathogen on the plant surface.

Studies on the phyllosphere microflora of several plants in relation to incidence and development of plant diseases have been carried out by a large number of workers during recent years (Sharma and Gupta, 1979, on brown sarson; Khara and Singh 1981, on tomato; Gupta et al., 1983, on chilli; Kathur et al., 1985, on sunnhemp). Most of these observers have found significant variation in the distribution of microflora in relation to climatic condition, plant age, maturity of leaves and variety.

Distribution pattern of microflora on the two surfaces of leaves have been studied by Last and Deighton (1965) on Phyllanthus discoidens, Lindsey and Pugh (1976) on Hippophae rhamnoides and Thakur et al. (1978) on Azadirachts indica. They have reported significant variation in the composition as well as population of microflora on the two surfaces.

Reports on the air spore studies of different crops (Sharma and Gupta, 1978, over a field of brown sarson; Verma and Kamal, 1982, over arhar field; Gupta et al., 1983, over chilli field) reveal that the composition and population of air spora varies from crop to crop, from season to season.

Although sufficient studies have been made on the phyllosphere microflora of potato plants, very little work have
been done on the subject in relation to incidence and development of *Phytophthora infestans*. Kumar and Gupta (1976 a, 1976 b) studied the phyllosphere microflora of three potato varieties namely Kufri-Sinduri (moderately resistant), Kufri-Chandramukhi (moderately susceptible) and Kufri-Sheetman (susceptible) in relation to the incidence of *Alternaria solani* and reported qualitative as well as quantitative changes in the population of microorganisms in relation to variety, maturity of leaf, age of plant and climatic conditions. Kumar and Singh (1981) reported that autumn crop of potato was infested with a significantly higher number of fungal propagules as compared to spring crop. Bombawale and Bedi (1982) in their studies on the epidemiology of early blight of potato in Punjab, reported varying early blight intensities in the autumn and spraying crops of potato cultivars Kufri-Chandramukhi and Kufri-Jyoti.

It is obvious that the intensity of microorganisms on the phyllosphere varies from crop to crop and place to place, depending on the prevailing climatic conditions. In the plain districts of Assam, potato is grown from the month of October to March. During the crop season the plants are exposed to varying climatic patterns. There is a gradual decrease in temperature and increase in humidity up to January which is followed by increase in temperature and decrease in humidity during latter period of crop season. It is therefore interesting to study the distribution pattern of microflora together with *P. infestans* on the aerial surface of potato plants in
the fluctuating climate during the crop season.

The present investigation deals with the nature and composition of the surface and air microflora of potato crop, incidence and spread of *P. infestans* and seasonal variation of surface colonizers in relation to climatic factors.

**Materials and methods:**

**Cultivar:** The investigation was carried out on two field plots measuring 100 X 60 feet of two potato cultivars namely Kufri-Jyoti (resistant) and Up-to-date (susceptible), during the crop season 1983-84, in the district of Lakhimpur, Assam.

**Sampling:** Leaves were collected from different sites of the plots at random to make one composite sample for each treatment. Collections were done once in a month during November (moderately cold humid), December and January (cold humid), February (moderately cold humid) and March (moderately warm humid).

**Culture media:** Martin's 'Peptone-Rose bengal-Agar' Medium (Martin, 1950) was used for the determination of total fungal population. Bacterial population was determined by using 'Soil extract-Agar' medium (Allen, 1957). Total population of actinomycetes was determined by using 'Starch-Ammonium-Agar' medium (Kuznetsov and Arjunarao, 1972). 'Limabean-Agar' medium was used for determination of total population of *P. infestans*.
Phylloplane microflora of young and old leaves: The microflora were isolated from the leaf surfaces by 'leaf-washing technique' adopted by Kumar and Gupta (1976). Young and old leaves, collected from the plants of the two cultivars were cut into pieces measuring 10mm X 10mm size. Ten such pieces of leaves were washed in 100 ml sterile water, by shaking with a mechanical shaker, separately for each lot. One ml sample from each lot were poured in Petri-dishes containing 15 ml culture media and rotated for uniform distribution over the surface. The Petri-dishes were then incubated for 7 days at 28° ± 1°C and observed for the growth of the microorganisms. For the study of population of *P. infestans*, Petri-dishes were incubated at 20° ± 1°C. Three replications were made for each treatment. Results were expressed as no. of colonies per 100 cm² area of leaf surface.

Fungal population on two surfaces of leaves: Isolation of fungi from adaxial and abaxial surfaces of leaves were done by 'leaf-imprint' method adopted by Garg and Sharma (1963). Ten pieces of leaves measuring 10mm X 10mm in diameter of same age, collected from potato cultivar Up-to-date, were momentarily and gently pressed against solid sterile 'Peptone-Agar' medium in separate sterile 9 cm Petridishes. Three replications were made for each for adaxial and abaxial surfaces. The dishes were incubated for 7 days and observed for the occurrence of fungi. For *P. infestans*, 'Limabean-Agar' medium was used and incubated at 20° ± 1°C. Results were expressed as number of colonies per 100 cm² area of leaf surface.
Population of air microorganisms over potato field: The estimation of microorganisms, present over potato field of cv. 'Up-to-date' was done by 'Culture plate' method adopted by Kumar and Gupta (1976) and Sharma and Gupta (1978). Petri-dishes containing different culture media were exposed for 5 minutes at the heights of 2, 18, 34 and 50 inches from the ground. The exposed dishes were incubated for 7 days and observed for colonies of microorganisms. Observations were made once in a month during afternoon hours. Results were expressed as number of colonies per 100 cm² area of exposed dishes.

Results:

Studied on phylloplane microflora of young and old leaves of potato:

Results presented in Table II and Figure 35 show that a total of 20 species of fungi namely Aspergillus sp.I, Aspergillus sp.II, Penicillium sp.I, Penicillium sp.II, Nucor sp.I, Nucor sp.II, Epicoccum sp., Cladosporium sp., Fusarium sp.I, Fusarium sp.II, Alternaria sp.I, Alternaria sp.II, Cer-cospora sp., Trichoderma sp., Curvularia sp., Chaetomium sp., Rhizopus sp., Micropora sp., Verticillium sp. and Phytophthora infestans were present on the leaf surfaces of the two cultivars along with 3 isolates of bacteria and 2 actinomycetes. Among these, Aspergillus sp.I, Aspergillus sp.II, Peni-cillium sp.I, Nucor sp.I, Cladosporium, Rhizopus, bacteria isolate I and actinomycetes isolate I were found throughout
FIG. 35: POPULATION OF FUNGI, BACTERIA AND ACTINOMYCETES (COLONIES/cm²) IN THE PHYLLOPLANE OF YOUNG AND OLD LEAVES OF POTATO DURING THE PERIOD OF NOVEMBER TO MARCH 1983-84.
the crop season irrespective of cultivar and maturity of leaves. *Alternaria* sp.II, *Epicoccum*, *Trichoderma*, *Cercospora* and isolate II of actinomycetes were isolated from the leaves only up to February. *Penicillium* sp.II, *Fusarium* sp. II, *Alternaria* sp.II, *Epicoccum*, *Cercospora*, bacterial isolates II and III were found during November while *Penicillium* sp.II, *Mucor* sp.II, *Verticillium* and bacterial isolate III could not be isolated during the month of March.

Significant variation in the distribution of microflora in relation to season, variety and maturity of leaves were noted in the present investigation. Total population of microorganisms gradually increased with the advance in season and reached peak during January and gradually decreased during February and March.

The cultivar Up-to-date showed higher population of microorganisms throughout the season than Kufri-Jyoti (1340.81 colonies per 100 cm² in the leaves of Up-to-date as compared to 1045.38 colonies per 100 cm² in Kufri-Jyoti) (*Figure 34*).

The old leaves of both the cultivars contained significantly higher population of microorganisms than the young leaves irrespective of cultivar and plant age (1360.25 and 1027.45 colonies per 100 cm² in old and young leaves respectively).

Among the total microbial population per 100 cm² area of leaf surface, fungi were the most dominant (1054.0 colonies) followed by bacteria (160.68 colonies) and actinomycetes
RESULTS ARE EXPRESSED AS NO. OF COLONIES/100 CM².
(23.68 colonies per 100 cm² area). Considering the population of each microorganism per 100 cm² irrespective of plant age and variety, the bacterial isolate I showed the highest population (115.00 colonies) next in the order being *Gladosporium* (106.65 colonies), *Fusarium* sp. I (73.5 colonies), *Penicillium* sp. I (72.00 colonies), *Aspergillus* sp. I (70.16 colonies) and *Hueor* sp. I (69.34 colonies).

*P. infestans* was isolated from the leaves of both cultivars during February and March, maximum being during February. The fungus, however, was isolated in low proportion from the young leaves of *Up-to-date* in January. Population of the fungus was significantly higher in the leaves of cv. *Up-to-date* than Kufri-Jyoti throughout the season (total population on the two cultivars are 61.67 and 29.01 colonies per 100 cm² respectively). Further the old leaves of the plants of both cultivars showed slightly higher population of microorganisms than the young leaves (46.67 colonies) and 44.01 colonies per 100 cm² on old and young leaves respectively).

Studies on fungal population on two surfaces of potato leaves:

Results presented in Table LIII and Figure 37 show that the adaxial surface contained significantly higher number of fungal propagules than the abaxial surface during early stages of plant growth (763.6 and 380.2 colonies per 100 cm² on adaxial and abaxial surfaces respectively) in November, when the plants were 30 days old. The difference gradually decreased...
Total fungal population (no. of colonies per hundred cm²) on abaxial and adaxial surfaces of potato leaves during the period from November to March, 1983-84.

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Av.Ad : Average adaxial  Ad : Adaxial surface  
Av.Ab : Average abaxial  Ab : Abaxial surface
FIG. 97: POPULATION OF FUNGI (COLONIES/100 CM²) ON ADAXIAL AND ABAXIAL SURFACES OF LEAVES OF POTATO.

- Adaxial Surface
- Abaxial Surface
with age of plants. In March, when the plants were 150 days old, very little difference in the population on the two surfaces was found (863.5 and 806.7 colonies per 100 cm² on adaxial and abaxial surfaces respectively). The average population of fungi on adaxial and abaxial surfaces throughout the season were 1106.8 and 857.98 colonies per 100 cm² area respectively.

All the fungi except Fusarium sp. I and Rhizopus, showed higher population on adaxial surface than abaxial. The two fungi were found to be prominent on the abaxial surface (66.0, 73.3 and 69.3, 62.0 colonies per 100 cm² on adaxial and abaxial surfaces respectively of Fusarium and Rhizopus). Chaetomium, Nigrospora and Verticillium were not found on the abaxial surfaces till the plants were 90 days old.

Results show that variation exists in the distribution of P. infestans on the two surfaces. The fungus was isolated in higher proportion from the adaxial surfaces throughout the period of its persistence on the surfaces (60.36 and 44.66 colonies per 100 cm² on adaxial and abaxial surfaces respectively). During January the fungus could not be isolated from the abaxial surface.

**Studies on the population of air microorganism over a field of potato:**

Results presented in Table LIII and Figure 35 and 36 show the presence of 24 species of fungi viz. Aspergillus sp. I, Aspergillus sp. II, Penicillium sp. I, Penicillium sp. II,
FIG. 38: POPULATION OF FUNGI, BACTERIA AND ACTINOMYCETES (COLONIES/100 CM²) IN THE AIR OVER A FIELD OF POTATO DURING THE PERIOD FROM NOVEMBER TO MARCH 1983-84.
Fig. 39. Population of fungi, bacteria and actinomycetes at different heights in the air over a field of potato. (Results expressed as no. of colonies/100 cm² area.)
Mucor sp. I, Mucor sp. II, Cladosporium sp., Fusarium sp. I, Fusarium sp. II, Alternaria sp. I, Alternaria sp. II, Cercospora sp., Trichoderma sp. I, Curvularia sp., Chaetomium sp., Epicoccum sp., Nigrospora sp., Rhizopus sp., Trichoderma sp. II, Verticillium sp., Trichothecium sp., Dreschlera sp., Rhizoctonia sp. and P. infestans along with three isolates each of bacteria and actinomycetes in the atmosphere. Among these Aspergillus sp. I and II, Penicillium sp. I and II, Mucor sp. I and II, Cladosporium, Curvularia, Rhizopus, Bacteria I and Actinomycetes I were present in the atmosphere throughout the season and at all the heights under investigation. Fusarium sp. I was present throughout the season at all the heights except 34 inches during March while Cercospora sp. was isolated at all but 50 inches during January. Fusarium sp. II, Alternaria sp. I, Rhizoctonia and bacterial isolate II, were not recorded in air over the field during November. Alternaria sp. II, Nigrospora and Trichoderma sp. II were present only upto January while Dreschlera and isolate III of Actinomycetes were found upto February. Verticillium was found from December to February while Epicoccum was present in November and December. Trichoderma sp. I and Trichothecium made dominant appearance from February, the former, however, was isolated in low proportion in November upto 34 inches height. Chaetomium was isolated during December but not recorded in January. The fungus reappeared in the air in February and March. Bacterial isolate III was not found in November and December. Actinomycetes isolate II was not found in February.
and isolate III in March. Results show that fungi dominated the population of microorganisms in the atmosphere (1032.74 colonies per 100 cm²), next in the order being bacteria (216.01 colonies per 100 cm²) and actinomycetes (60.64 colonies per 100 cm²). Considering individual population of microorganisms irrespective of season and height, bacterial isolate I showed the highest population (105.17 colonies), followed by Cladosporium (94.83 colonies), Penicillium sp. I, (88.16 colonies), Mucor sp. I (87.33 colonies) and Penicillium sp. II (76.83 colonies per 100 cm²). The population index of other organisms fell between 5.16 and 69.16 colonies per 100 cm².

Figure 38 indicates that total population of microorganisms gradually increased with the advance in season reaching the peak population in the month of February (1639.36 colonies per 100 cm²). The intensity of population dropped to minimum during January which was particularly evident for fungi. However, bacteria and actinomycetes continued to increase with the advance in season.

Results also indicated that population varied according to the plant height from ground level. Maximum population was obtained at a height of 18 inches above ground (1662.74 colonies) followed by 2 inches (1487.36 colonies). Population gradually decreased with increase in height (1257.60 and 829.0 colonies at 34 and 50 inches respectively). Bacterial isolate I dominated at 18 inches height (149.34 and 147.34 colonies respectively) while fungi dominated at higher
levels. At the height of 34 inches above ground *Gladosporium* showed highest population (100.62 colonies) while *K. coron* sp. I dominated at 50 inches (75.32 colonies per 100 cm²).

*Phytophthora infestans* was abundant in the atmosphere during February and March (92.35 and 55.32 colonies). The fungus, however, was found in low proportion in December and January (8.32 and 5.0 colonies respectively). Total population of the fungus in the air over the plot throughout the season was 30.96 colonies per 100 cm² area. The spores of the fungus were more abundant in the lower heights than higher. Maximum population of *P. infestans* was recorded at the height of 18 inches from the ground (63.34 colonies) and minimum at 50 inches (11.34 colonies per 100 cm² area).

**Discussion:**

In the present investigation on phyllosphere microflora of the two cultivars of potato, varying in susceptibility, incidence of phyllosphere microflora was found to be dependent on several environmental factors such as plant age, maturity of leaves, variety and climatic conditions.

Different stages of plant growth had a marked influence on the population and composition of microflora. The population increases with the age of plants and maturity of leaves. Hollomon (1967) working on potato in Australia reported a significant rise in spore density with the age and maturity of the host plants. Kumar and Gupta (1980) studying the aero-
biology of *Alternaria solani* in relation to phyllosphere of potato reported increase in conidial population in the air and reached peak when the crop was quite old and mature. Similar observations were made by a number of workers for different crops (Thakur et al., 1978; Khura and Singh, 1981; Verma and Panal, 1982). The number of fungal colonies has been reported to increase in older leaves of *Crotolaria juncea* (Thakur et al., 1985) and *Sesamum* and *Rosipium* (Sharma and Kukherji, 1976). The results of the present investigation on two potato cultivars stand in conformity with findings of the above workers. The increased number of microorganisms in the matured leaves may be due to more exposure period of these leaves which resulted in higher deposition of microorganisms as compared to the young leaves.

Results of the present investigation indicate that climatic factors profoundly influence the distribution of microflora on the leaf surface. The plants were densely populated with microbes in the cold humid season, maximum being in the month of January. The comparatively low temperature (max. 21.06 and min. 7.63°C) and high humidity (82.61 and 79.70 percent during 0630 and 1750 hours respectively) might have favoured sporulation on the surfaces as suggested by Kumar and Gupta (1976) and Kumar and Singh (1981). The significant decrease in the population on the leaf surface as well as composition of mycoflora during the month of March may be attributed to increasing temperature (max. 27.51°C, min.
16.78°C), and decreasing humidity (0830 hrs. 74.09 percent; 1750 hrs. 69.70 percent) which were unfavourable for the organisms.

The host variety is another factor that influences the population, composition and also the nature of phyllosphere microorganisms. Sharma (1971) noted that susceptible variety of sorghum was densely populated than the resistant variety. Kumar and Gupta (1976) working on potato found that susceptible Kufri-Sheetman variety carried the highest population of microorganisms in contrast to the moderately susceptible Kufri-Chandramukhi and moderately resistant Kufri-Sinduri cultivars of potato. In the present investigation the susceptible Up-to-date variety contained more microorganisms than the resistant Kufri-Jyoti, which supports the findings of the above workers. The difference in phyllosphere in relation to host variety may be due to variations in the nature and composition of leaf leachates of different varieties (Kumar and Gupta, 1976), besides biochemical and anatomical aspects of host plants.

It is further evident from the present investigation that the population of microorganisms on leaf surface varies according to the maturity of the leaves. The older leaves supported higher population than the younger leaves irrespective of host variety. Similar results have been reported by Kumar and Gupta (1976) and Khara and Singh (1981). Sinha (1971) working on leaf surface microflora of four solanaceous plants stated that the gradually increasing number of fungi may reflect the increasing deposition from the air spora during prolonged
exposure or it may be due to multiplication of microorganisms in the phylloplane of old leaves. He further stated that as larger amounts of nutrients were leached from ageing leaves than from young leaves, the exudates of old leaves, there was increased colonization by saprophytic microorganisms; This resulted in specific association and increase in abundance of the microfloral population.

Variation in the distribution pattern of microorganisms on the two surfaces of leaves was observed in the present investigation. The adaxial surfaces of leaves carried higher population of microorganisms than the abaxial surfaces. Sharma et al. (1974) and Garg and Sharma (1983) also isolated higher population of fungi from adaxial surfaces of Lantana camara and Triticale and Guar leaves respectively. Pugh and Buckley (1971) observed that Sporobolomyces roseus was present in greater number on upper surfaces of Sycamore leaves, whereas, Cladosporium herbarum occurred most predominantly on lower surfaces of leaves. Last (1970), however, isolated colonies of Sporobolomyces roseus, Cladosporium herbarum, Epicoccum purpureascens, Alternaria alternata, Cephalosporium acremonium and Fusarium spp. more frequently, from upper surfaces of Hippophae rhamnoides. Bainbridge and Dickinson (1972) observed more extensive growth of yeast and hyphal forms on abaxial surfaces of potato leaves. This difference, however, dissipated on senescent leaves. The extensive growth of Alternaria, Aureobasidium, Cladosporium and Epicoccum was attributed by him to rough texture of leaves and greater leaching of nutrients from their adaxial
surfaces. In the present investigation, all fungi except *Fusarium* sp. I and *Rhizopus* showed better growth on adaxial surfaces. These two fungi were found to be more on the abaxial surface.

*P. infestans* was not found on abaxial surface during January. The fungus however was isolated from both surfaces during March. The adaxial surface showed higher population of the fungus than abaxial surface.

The variation in the distribution pattern of microflora on the two surfaces of leaves have been attributed, in some cases, to possible morpho-anatomical characteristics of leaf surfaces and to nature and amount of leaf leachates. Garg and Sharma (1986) stated that more growth of fungi on the adaxial surface of triticale leaves might be due to higher pollen densities on the surface. They further suggested that extent of exposure of leaf surface may be another factor of unequal distribution of microflora on the leaf surface. The adaxial surfaces are directly exposed to atmosphere and it might help higher deposition of spores from the atmosphere.

Air spora studies over the potato field revealed considerably higher population of microflora in the air than on the leaf surfaces. In addition to the microflora found in phylloplane, the air contained few more organisms such as *Trichoderma* sp. II, *Trichotheclum* sp., *Rhizoctonia* sp. and isolate III of actinomycetes. The decrease in the population of microflora during January may be attributed to low temperature and high humidity prevailing during the period, which had an adverse
effect on the air spora, as suggested by Sharma and Gupta (1978). It is obvious that high humidity in the atmosphere had forced the spores to settle on the ground or on the surface of plants which is further evident from the maximum population of microflora on the phylloplane during January. The decrease in the quantity of air spora during March may likewise be related to the gradual decrease in humidity with increase also in temperature. The high wind/might have blown out most of the microorganisms preventing the total deposition on culture plates exposed over the potato field.

Considerable variation in the distribution of microflora at different heights was evident in the present investigation. A gradual decrease in the population of microflora from lower to higher heights was observed. Differential pattern of distribution of air spora at different heights was also reported by Baruah and Bora (1982) on their studies of air spora over some angiospermic plants. In the present investigation, bacteria were found to dominate the air in an around the crop while fungi dominated at higher heights. This may be due to better buoyancy of the fungal propagules. Overall population of microorganism was maximum at lower heights which is attributable to the gravitational force exerted upon the spores.

*Phytophthora infestans* was isolated from the air over the crop during the periods from December to March. Maximum population of the fungus was observed during February which coincides with the extreme development of late blight disease in the crop. It is further interesting to note that the fungus
was detected in the air well before its occurrence on the leaf surface. It is thus obvious that the spores of the pathogen were carried by air current from some foreign sources which served as the primary inoculum on the leaf surface. Once the pathogen gets established on the host surface, more and more sporangia were produced which is evident from the sudden increase in population of the fungus during February and March.

Results obtained in the present investigation indicates that the incidence of *P. infestans* on the potato crop may also be influenced, to some extent, by other microorganisms. During the month of January, when the leaves showed maximum population of microflora, *P. infestans* could be isolated only from the young leaves of the susceptible cultivar. It is interesting to note that few fungi such as *Trichoderma*, *Aspergillus*, *Penicillium* and *Fusarium*, which are reported to be inhibitory to *P. infestans* (Lacey, 1965), were present in abundance on the leaves of both resistant and susceptible cultivar, during the period. There is, thus, reason to believe that these fungi might have restricted the growth of *P. infestans* on the leaves preventing further development. This view is further supported by the fact that population of other microorganisms decreased with complete disappearance of *Trichoderma*, *Alternaria* sp. II, *Epicoccum*, *Cercospora* and actinomycetes isolate II. Thus adverse effect of phylloplane saprophytes may also be considered as a factor on the incidence and development of *P. infestans*, along with environment and host factors.

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