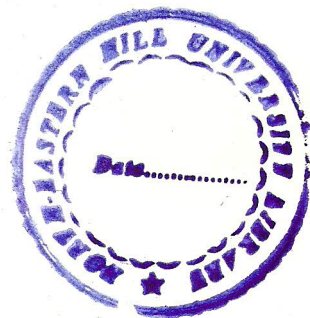


STUDIES ON SEED MYCOFLORA OF
Phaseolus vulgaris Linn.

BY

ATANU BHATTACHARJEE



THESIS SUBMITTED
IN FULFILMENT OF THE DEGREE OF
DOCTOR OF PHILOSOPHY IN BOTANY

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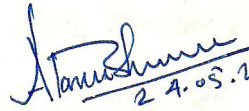
CERTIFICATE

I, Atanu Bhattacharjee, do hereby declare that the subject matter of the thesis entitled "Studies on seed mycoflora of *Phaseolus vulgaris* Linn." is the record done by me and that the contents of this thesis did not form basis of the award of any previous degree or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the North-Eastern Hill University for the degree of Doctor of Philosophy in Botany.


26/5/01
(Head of Department)


24.5.2001
(Supervisor)


24.05.2001
(Candidate)

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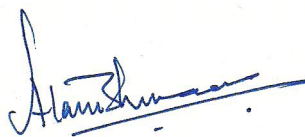
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INTRODUCTION

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INTRODUCTION

Common bean (*Phaseolus vulgaris* Linn.) is an important leguminous crop that provides a large part of the daily protein requirement of the people residing in South America, the Caribbean, Africa and Asia. Common bean is a principle source of protein for more than 500 million people in Latin America and Africa; when consumed as snap beans, it is an important dietary source of vitamins and minerals in Asia. A vast stretch of land devoted to bean production in developing countries has increased steadily in the last couple of decades (CIAT, 1992). However, production of beans has not kept pace with the ever increasing population and it must increase to a level of 42% in Latin America and 72% in Africa by the year 2000 so as to satisfy the expected demand (Janssen, 1989). In China alone, the demand for snap beans was estimated to be 3.5 million tons in 1989 and 4.3 million tons is projected for the year 2000 (Yan, *et al.* 1995). Bean production in developing countries is often on marginal land and few developing countries have significant reserves of arable land available for bean cultivation. An increased bean production would largely have to come through yield per hectare rather than expansion of area under cultivation. Average bean yields in most developing countries are below maximum yield potential, which indicates that substantial improvement in bean production could be realized by increasing yields per unit land area. Average yields of common beans are less than 1ton / ha in most developing countries (Laing *et al.*, 1984). The main reason for low yields are water

deficiency, high incidence of diseases, insects and use of inorganic fertilisers. However, there is a general dearth of information pertaining to the various aspects of bean cultivation.

P. vulgaris Linn. is the most widely grown of the five cultivated species of *Phaseolus*. The other four species are *P. coccineus*, *P. lunatus*, *P. polyanthus*, *P. acutifolius* and *P. latifolius*. *P. vulgaris* L. is also known as Dry, Field, French, Snap, Navy, or Kidney beans. Beans are usually grown in tropical countries for dry seeds and in temperate countries for dry seeds as well as for fresh pod consumption and for processing as frozen vegetables.

It is now well known that seeds play a vital role in the introduction of plant pathogens into new areas. The pathogens retain their potentiality till they are successful in finding a specific host for infection and subsequent disease development. Nowadays, host specific mutant strains are developing. Hence, the entire mankind faces an uphill task of saving the crop and subsequent increase in its production.

As early as 800-900 years ago, in summer, in the Eastern Mediterranean, man began to depend on seeds as a principal means of carrying his main crops, the cereals, from place to place and from season to season. The pathogens were carried with the seeds from the beginning, the earliest confirmed record of this association being from Jarmo, about 800 years ago (Stewart and Robertson, 1968). The nine major important crops of the world are wheat, rice, maize, barley, sorghum, sugar beet, common bean (*P. vulgaris*), soybean and groundnut, which represent the greater part of the food production of the world. Approximately 90% of all food crops grown on earth are propagated by seed (Neergaard, 1977). Hence, now it is pertinent to say that crops of

high yielding variety and disease resistance are the need of the hour to sustain the volatile population of the globe. But, then all these efforts could go in vain if the growers were not made aware to use the disease free and high yielding variety of seeds. Though the importance of obtaining quality seeds has been realised in the past, still, even in this 21st century, we cannot be completely satisfied with the extent of seed research. There is no doubt that the scientists all over the world has been working tirelessly and their efforts are reflected through the technological development of the seed industry.

Microorganisms exhibit their existence by harboring the seeds both externally as well as internally. The microorganisms become active under favorable environmental conditions such as light, temperature, moisture content etc. Among the microbes, fungi play a significant role in determining the quality and longevity of the seeds (Christensen and Lopez, 1963; Christensen and Kaufman, 1968 and Christensen and Mirocha, 1976). Hence, it becomes quite evident that it is necessary to test the health of seeds prior to sowing in order to ensure a better crop production.

Avoiding crop failures and the use of high yielding cultivars are the two main ways of boosting the crop production. But, even after using high yielding variety, the entire painstaking process may be futile if the microbes are able to invade the seed material. The disease caused by the microbes may be responsible for about 10% loss in major crops in India.

In the context of present day agriculture, sharing of seed and other propagative plant material has become essential for improving crop productivity. It is a known fact that India is one of the leading producers of many economically important crops, hence,

it is obvious that our country imports many varieties of seeds and plant materials to meet the demand of the ever-increasing population. So it is quite likely that along with the imported materials, some virulent strains of fungi may sneak in and cause devastating diseases, which may even take the form of an epidemic. It is due to this problem that imposition of plant quarantine have become essential and at present this law has been imposed in many countries. The main objective of this law is to prevent the entry of serious disease causing microbes into a country or a territory.

More and more attention is now being paid to the microbes causing disease and their ability to produce toxins. The deterioration in quality of seeds may be defined as, "the falling from a higher to a lower level in quality, character or vitality". It implies the impairment of vigour or usefulness (Gove, 1965).

The mould fungi cause extensive damage to the seeds. They grow well in grains and seeds during storage. They also may impair or retard the growth of seedlings. The impairment is caused mainly by the toxic metabolites secreted by the microbes. The quality of the seeds also deteriorate following the invasion by the microbes.

Temperature is another important factor that determines the type of microorganisms that will grow on the seeds during storage. At a temperature of about $25 \pm 1^{\circ}\text{C}$, almost all types of fungi are predominantly available. *Aspergillus flavus*, which produces the fungal metabolites, is the most predominant. It is also reported to be carcinogenous (Wogen, 1965). At low temperature, however, most fungi are found to be dormant. Above 50°C , most fungi are killed. The deterioration of seeds may occur due to abrupt changes in the respiratory pathway and metabolic activities.

The seeds and their by-products are the major components of the diet of man and a large number of farm animals. At the global level, cereal grains contribute about 50% of the per capita energy intake (Fao, 1993).

The seeds remain viable for many years. Seeds of crop plants with low moisture content remain viable for longer time periods when stored under low temperature. The longevity of seed-borne pathogens can be independent of the seeds they inhabit and depends on the capability of the pathogen to remain viable as well as virulent from one season to the next in or on seeds. Pathogens may live longer than the seeds they colonize, for example *Colletotrichum lindemuthianum* remained active after the bean seeds, it colonised, lost their viability (Laing *et al.*, 1988). Neergaard (1984) generalised that (i) hyaline fungi with strong pigmentation and thick conidial walls were fairly long lived; (ii) fungi with fruiting bodies such as acervuli and pycnidia remained viable for long time periods, (iii) smut fungi lived long and (iv) fungi with thick walled resting mycelia survived the longest.

Airtight containers favour the survival of the seed-borne pathogens. Dry and cool weather provide an ambient condition for growth of seed-borne inoculum. The germination of *Phoma rabiei*, threshed after 5 months of storage at 25^o to 30^oC, was found to be 50%. *C. graminicola* survived in maize kernels for more than 3 years at 4^oC. Advances in technology enhanced the ability of man to successfully store harvested plant products. Containers made of plant and animal materials were probably in widespread use during the last ice age; however, due to their perishable nature none of them survived. Basket making was highly developed art by 7000 BC. Earlier, baskets were used for gathering seeds, nuts, fruits and other vegetable materials and for storage

until their subsequent use. The ability to make pots of fired clay was an extremely significant advancement in storage technology and its appearance coinciding with the first domestication of grain bearing plants cannot be considered a mere chance. For the first time, it allowed the stored products to be sealed in containers that would significantly decrease the chance of loss due to fungal infestation. Another early technique developed for the storage of grains was underground pits and silos. During storage, respiration of the grain decreased the oxygen concentration and elevated the carbon dioxide level tremendously, thereby decreasing the chances of losses due to rodents and insects. Bernard in France conducted the first scientific studies on modified storage atmosphere in 1819.

The storage fungi may induce a decrease in germination percentage, important in malting barley and in seeds to be used for planting; discolored or otherwise damaged embryos or whole kernels, may help to determine the grade and price of the grains fit or unfit for consumption. Toxins constitute a health hazard for man and animals. They also cause heating, which is accompanied by drastic reduction in quality of seed or their complete spoilage (Christensen and Kaufman, 1965).

Forgac and Carll (1962) summarized information on a number of mycotoxins and animal disease caused by ingestion of foods and feeds invaded by fungi that produce toxins. Some of these mycotoxins present in common and widespread fungi result in severe illness and death of the consumers. The fact that fine quality seed is the need of the hour has been realised by the ancient growers since the time of cultivation. Though we are now in the 21st century, when we turn back to see the achievements, we find that the progress is still far from satisfactory. The scientists from all over the world

have been engaged in the seed research and some of their effort has been paid off as is seen in the advanced growth of seed industry.

There are a wide variety of microbes that are associated with the seeds. Under favourable conditions these microbes become active and cause severe damage to the field crops. Amongst these microorganisms, fungi play a significant role in determining the quality and longevity of seeds (Christensen and Kaufman, 1965 and Christensen and Mirocha, 1976). Hence, seed health testing and the use of healthy seeds are of great significance in improving the condition of the agricultural products.

It is known that many seed-borne fungi produce toxins, which at times may act as carcinogens. They also act as disease carriers, which may prove to be fatal to many crop plants. The change occurring in the seeds are not reversible and moreover, the qualities of the seeds degenerate even after full maturation.

While cleaning the seeds during storage, most of the unwanted materials are removed. However, some fungi still stay on and cause seed deterioration. The ideal temperature at which saprophytic and parasitic fungi can regain their germinating ability is 14°C in cereals. The fungi included in this group are the species of *Alternaria*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Penicillium*, *Aspergillus*, *Rhizopus* and *Helminthosporium*. These fungi are termed as storage fungi by Christensen and Kaufman (1965) which separates them from those of the field fungi, that may be present in the seed before or after the seed storage and destroy the seeds. The stored fungi may flourish under dry conditions. These fungi have the capability of attacking any crop under a favourable climatic condition.

The storage fungi also have the capability to alter the level of fatty acids and non-reducing sugars, induce a foul smell and offer discolourisation of seeds. Species of *Aspergillus* and *Penicillium* are the two most common fungi that cause extensive damage to the stored seeds throughout the world. The spoilage of the seeds takes place at 45°C at 65-100% relative humidity. The activity of the fungi depends to a large extent on the physical condition, vitality and moisture content of the seed, temperature and relative humidity of the storage atmosphere.

The available stored materials and the storage tissue may be reduced by the decaying activity on seed by the saprophytic microorganisms thus making the crop susceptible and they may also affect the seedling germination. When the seeds from such seedlings are sown again, the microorganisms regain their growth under favorable conditions that may ultimately spoil the seed.

The toxic materials released from certain fungi affect the seedling growth severely. The appearance of the seedlings is also altered. The microorganisms affect the rise in temperature of the stored seeds (Panasenko, 1967).

The saprophytic mycoflora interrupts in the seed certification and indexing process, which determine the presence of the pathogens. The saprophytes are permitted to grow when the seeds are incubated and so they are the main reason for the difference in seed tests for the presence of plant pathogens (Limonard, 1967 and 1968).

In adverse field conditions, the mycelium of many fungi remain dormant and hence, are unable to impair seedling development (Mathur and Hansing, 1962).

Rise in temperature due to the excessive growth of fungi on stored seeds is usually noticed with 18% moisture content, in case of stored cereal seeds, the

temperature shot up from 17^o to 43^oC (Gilman and Barron, 1930). Species of *Aspergillus* and *Penicillium* are the two main dominant fungi associated with the rise in temperature (Wallace and Sinha, 1962)

Annually a large quantity of crop is being lost or spoiled due to fungal infection both in terms of quality as well as quantity. The commercial value of the seeds gets depleted considerably and sometimes the grain is not fit for human consumption. The most common diseases encountered include (i) reduced size of the seed, (ii) discolourisation, (iii) *Fusarium* root rot, (iv) anthracnose, (v) necroses of seed and (vi) other physiological changes occurring in the seed.

Among the group of fungi, the species of *Fusarium* is the most dangerous customer to the crop plants. The common diseases caused by the pathogens include stunted plant growth, root rots and wilt. *Alternaria* in French bean causes leaf spots. The species of *Colletotrichum* is another successful pathogen, which establishes a firm hold on the leaf and pods of the crop. This pathogen is also responsible for decomposition of the seeds and the plant as a whole. The symptoms include dark brownish spots, which enter the pods and decompose it giving a bad odour. Considerable loss in yield of the crop is recorded every year due to such activity of the pathogen. Sometimes the loss in yield becomes difficult to ascertain.

With the infection of the seed, there seems to be some changes in their biochemical aspects. The storage materials, enzymatic activity and respiratory pathway all get distorted from the normal range. Their sites and mode of action still need a careful and thorough attention.

Having a glance at the volatile population of the globe, it becomes an obvious fact that the demand for food supply has to be increased to keep pace with the increasing population. Hence, it is an imperative need to make an in-depth analysis of the seed production regarding its yield and quality. Seed pathology and its study has, therefore, become an interesting and a main thrust area of present agricultural research. Neergard (1977) defined seed pathology as, “the science and technology dealing with (i) seed-borne plant disease, (ii) seed diseases, (iii) the mechanism of their transmission, (iv) factor influencing their development, (v) techniques for their detection in seed, (vi) the method for preventing and controlling these diseases in the field and during seed storage and (vii) the assessment of seed-borne inoculum and seed diseases for seed certification schemes, quarantine, planting value and quality for consumption or processing”.

ICAR, an important research agency is involved in extensive research work on seed and its development. In Northeast India, the ICAR is involved in developing hybrid seeds with care to ward off invasion by pathogens and its subsequent infection.

Considering the importance of fungi in causing the diseases in plants and reducing the yield of the field crops, the present investigation has been undertaken to generate important data for controlling the menace of fungal attack on bean seeds and increasing the yield for the ever- increasing population of the earth.

The following plan of work had been adopted to fulfill the desired goal of my research activity:

- (1) Collection of different varieties of French bean (*Phaseolus vulgaris* Linn).
- (2) Survey of seed borne fungi, their isolation and identification.

- (3) Comparative study of different storage practices with respect to the incidence of fungi.
- (4) Studies on the effect of certain seed-borne fungi on the germination of *Phaseolus vulgaris* Linn. seeds.
- (5) Studies on the impact of seed leachates on the germination of spores of certain seed-borne fungi
- (6) Studies on the effect of certain seed borne fungi in decomposition of the seed.
- (7) Studies on the influence of certain agro-chemicals on the seed-borne mycoflora.
- (8) Screening of seeds for aflatoxins.
- (9) Control of certain pathogens by biological means.