Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 7, No. (3) : 2005 : 577-582

SEED MYCOFLORA AND STORAGE EFFECT IN THE GERMINATION OF FRENCH BEAN (*PHASEOLUS VULGARIS* LINN.)

A. BHATTACHARJEE AND M.S. DKHAR*

*Department of Botany, School of Life Sciences, Permanent Campus, Shillong -793022, Meghalaya, India.

Key words : Containers, Fungi, Germinability, *Phaseolus vulgaris* Linn., Seeds, Storage.

Abstract- Local variety of French bean (*Phaseolus vulgaris* Linn.) viz Meghalaya has been selected for the present study. Of the three methods used for the isolation of fungal species, blotter method proved to be the most effective followed by agar method. The dilution plate method was least effective in harbouring the fungal species vis-avis the two methods. A total of 26, 28 and 28 fungal species were isolated by dilution plate, agar and blotter methods respectively. The fungal species such as *Aspergillus niger, Cladosporium cladosporioides, Colletotrichum lindemuthianum, Fusarium oxysporum, Pythium intermedium* and *Trichoderma viride* were found to be present in all the seasons of the year. The storage of bean seeds in different containers recorded maxmum number of fungal species in rainy season followed by summer and minimum was observed in winter season. The storage of seeds in earthen pots had a negative effect on the overall rate of germination. The impact of storage on seed was slightly better in bamboo basket followed by gunny bag. Seeds stored in iron bins exhibited a very low level of mortality. With increase in storage period, the viability of the seeds decreased and the germinability reduced. Significant variation in percentage fungal infection was observed between gunny bag and earthen pot and between earthen pot and iron bin at 0.05 level.

INTRODUCTION

It is a well known fact that the seeds play a vital role in 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. Approximately 90% of all food crops grown on earth are propagated by seeds (Neergaard, 1977). Hence, 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 all these efforts will go in vain if the growers were not made aware to use the disease free and high yielding variety of seeds. Avoiding crop failure and the use of high yielding cultivars are the two main ways of boosting the crop production. However, even after using high yielding variety, the entire painstaking process may be futile if the microbes are able to invade the seeds. The disease caused by the microbes may be responsible for about 10% loss in major crops in India.

Phaseolus vulgaris Linn. commonly known as French bean is the most widely grown species of *Phaseolus*. 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 bean has not kept pace with the ever increasing population. Bean production in developing countries is often on marginal land and a few developing counties 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 yield 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 area. Average yields of common beans are less than 1 ton/ha in most developing countries (Laing *et al.*, 1984). The main reasons for low yields are water deficiency, high incidence of diseases, insects and use of inorganic fertilizers. Microorganisms exhibit their existence by harbouring the seeds both externally as well as internally. Among the microbes, fungi play a significant role in determining the quality and longevity of the seeds (Christensen and Lopez, 1963; Christensen and Kaufman, 1969 and Christensen and Mirocha, 1976). It is necessaray to test the health of seed prior to sowing in order to ensure a better crop yield. The mould fungi cause extensive damage to the seeds. They grow well in grains and seeds during storage. They may also 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 deteriorates following the invasion by the microbes.

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

MATERIALS AND METHODS

Local variety of French bean (*Phaseolus vulgaris* Linn.) viz. Meghalaya has been selected for the present investigation. While drawing the samples from the bulk storage, international Rules for Seed Testing Association (ISTA, 1966, 1976) was followed. For the two-year survey of seed-born mycoflora, fresh samples were collected each soon after the harvest period and stored in the laboratory for each incumbent year. The survey of seed-borne mycoflora was conducted on a seasonal basis, taking three seasons (winter, summer and rainy) each year. The samples were stored in the laboratory and the working samples for periodical screening were drawn aseptically from the stock samples.

The three methods were followed for the isolation of fungi from *P. vulgaris* Linn. seeds. They were *(i)* Standard Blotter Method , *(ii)* Agar Plate Method and *(iii)* Dilution Plate Method. The Standard Blotter Method as recommended by ISTA (1966) with slight modification as suggested by Limonard (1966), known as the freezing method was followed. The infected seeds were later on transferred to potato dextrose agar medium (PDA) for confirmation and further investigation.

For the comparative study of different storage practices with respect to the incidence of fungi, four types of storage containers used for the present experiment were *(i)* gunny bag *(ii)* bamboo basket, *(iii)* earthen pot and (iv) iron bin. Three kilograms of seeds were kept in each of the the sterilised containers. The containers were then sealed properly and kept in the laboratory at room temperature for a period of 12 month. Every precaution was taken to prevent the possible attack by rats and mites during this period.

The samplings were done at 60 day intervals. The suitability of different containers, observation in respect of the incidence of fungi and the quality of the seeds were determined by *(i)* determining the qualitative and quantitative incidence of the fungi during different periods of storage, *(ii)* comparing the percentage infection of the seeds at the initial stage with the after storage, *(iii)* determining the fluctuation of moisture content of the seed in various containers during different periods of storage and (iv) determining the germinability of seeds in various containers during and after storage. Blotter method was used to test the germinability of the seeds. The Petri dishes were incubated at 20+1°C under the alternate cycle of light and dark. A stage had come when the seedlings had started to coil inside the Petri dishes. It is the stage that the dishes were uncovered and on the 11th day, the dishes were observed and the seedlings were removed one by one. The pre- and the post emergence mortality of the seedlings were counted and the percentage germination of the seeds were calculated.

RESULTS AND DISCUSSION

Table 1 depicts the list of fungi isolated from the seeds during the study periods. Of the three methods, blotter method proved to be the most effective in terms of the number of fungal species isolated followed by agar method which may be due to the availability of high moisture content in the blotter's plate and of fungal nutrients in the agar plate. This agrees with the findings of singh *et al.* (1984), Paul and Mishra (1992) and Neergaard and Saad(1962) who concluded that the blotter plate and agar plate methods are both valuable and supplementary to each other. The dilution plate method was least effective in harbouring the fungal species vis-a vis the two methods. A total of 26, 28 and 28 fungal species were isolated by dilution plate, agar and blotter methods respectively. Maximum fungal species were recorded in rainy season followed by summer and minimum was recorded in winter season. This could be due to the differences in the temperature and moisture conditions favourable for the growth of the fungal species. This is in conformity to the findings of Reddy and Reddy (1983) and Paul and Mishra (1992). The fungal species such as *Aspergillus niger, Cladosporium cladosporioides, Colletotrichum lindemuthianum, Fusarium oxysporum, Pencillium intermedium* and *Trichoderma viride* were found to be present in all the seasons of the year which may be due to their adaptability over a broad spectrum of nutritional and environmental conditions.

A. niger, Rhizopus nigricans, Penicillium spp, F. oxysporum and mycelia sterilia dominated the agar

Table 1. The list of fungi isolated from *Phaseolus vulgaris* Linn. seeds at different seasons

Fungal Species	Winter			Summer			Rainy		
	D	A	B	D	A	B	D	A	B
Aspergillus alutaceus	+	+	-	+	+	+	+	+	+
A. candidus	+	-	+	-	+	+	-	-	+
A. clavatus	+	+	+	+	+	+	+	+	-
A. flavus	+	+	+	+	+	+	+	+	+
A. niger	+	+	+	+	+	+	+	+	+
A. ruber	-	+	-	-	+	+	-	+	+
A. tenusi	-	+	+	-	+	-	-	+	-
Alternaria alternata	-	+	+	+	+	+	-	+	+
Cephalosporium acremonium	-	+	+	+	+	+	+	+	+
Chaetomium glosbosum	+	-	+	+	+	+	+	+	
Cladosporium cladosporioides	-	+	-	+	-	+	+	+	+
C. gressi	-	-	-	+	+	+	-	+	+
Colletotrichum lindemuthianum	+	+	-	+	+	-	+	-	+
Fusanium moniliforme	+	+	+	+	+	+	+	+	+
F. oxysporum	+	+	+	+	+	+	+	+	+
Mammaria echinobotryoides	-	-	+	-	+	+	+	+	+
Penicillium chrysogenum	-	+	-	+	+	+	+	+	
P. expansum	+	+	+	-	+	+	+	+	+
Penicillium sp.	-	-	+	-	-	-	-	+	+
Phoma medicaginis	+	+	-	+	+	+	+	+	+
P. pomogranatus	+	+	+	-	-	-	-	+	+
Phoma sp.	-	-	-	+	+	+	+	+	+
Pythium intermedium	-	-	-	-	-	+	+	+	+
Pythium sp.	+	-	-	+	+	+	-	+	+
Rhizopus nigricans	-	-	-	+	+	+	+	+	+
Rhizoctonia solani	-	-	+	+	+	-	-	-	+
Trichoderma viride	+	+	+	-	+	+	-	+	+
Trichoderma sp.	-	-	+	+	+	+	+	+	+
Sterile mycelia									
(I) Brown	-	+	-	+	+	+	+	+	+
(II) White	+	-	+	-	+	+	+	+	-

+=Present, -=Absent, D=Dilution plate method, B=Blotter method A= Agar method

plates. This finding corroborates with the work of Aulakh *et al*, (1976) and Paul and Mishra (1992) mainly because of their fast growth. It was observed that the number of fungal species decreased gradually with increase in storage period in both the varieties. This may be due to the varied fungal activity with increase in storage period .

Fig .1 shows the percentage fungal infection of the seeds during the storage period . The seeds stored in earthen pots showed a higher percentage of infection and low germinability (Table 2) which could be due to the ability of the post, to retain maximum moisture favourable for the fungal infection (Fig. 2). With increase in storage period the viability of the seeds decreased and the germinability reduced which may be due to the interference of the fungi in the metabolic activity of the seeds and the consumption of stored food matter resulting in the abnormal or no growth of the seedlings.

In some isolated cases poor germination and decaying of seeds were observed. This draws support from the findings of Kumar and Nema (1973) and Rati and Ramalingam (1974). Reduction in the germination can be explained , as put forward by Christensen and Kaufman(1965), that the fungal association changes the mitochondrial set up of the cell integrity and ultimately affect the viability of the seeds. This suppression of seed germination could be due to the release of gungal toxins thereby hampering the metabolic activities of the seed, whereby, there may be depletion of some metabolites and the abolition of others. This is in conformity with the observation of Mishra *et al.* (1979), Omokanye and Onifade (1993), Begnami and Cortelazzo (1996) and Vieira (1998). The inefficiency

Table 2. Percentage germination and mortality of *Phaseolus vulgaris* Linn. seeds during the storage periods in different containers.

Storage (Days)	GUNNY BAG			BAMBOO BASKET			EARTHEN POT			IRON BIN		
	Emergence Mortality (%)			Emergence Mortality (%)			Emergence Mortality (%)			Emergence Mortality (%)		
	Pre	Post	FS (%)	Pre	Post	FS (%)	Pre	Post	FS (%)	Pre	Post	FS (%)
0	13	2	85	13	2	85	13	2	85	13	2	85
60	20	6	74 (12.94)	26	3	71 (16.47)	20	6	74 (12.94)	20	4	76 (10.59)
120	25	10	65 (23.53)	50	4	46 (45.88)	33	8	59 (30.59)	40	6	54 (36.47)
180	30	12	58 (31.76)	55	7	38 (55.29)	54	12	34 (60.00)	45	8	47 (44.71)
240	45	15	40 (52.94)	60	8	32 (62.35)	60	14	26 (69.41)	54	10	36 (57.65)
300	60	15	25 (70.59)	70	11	19 (77.65)	66	17	17 (80.00)	62	12	26 (69.41)
360	75	20	5 (94.11)	74	14	12 (85.88)	75	18	7 (91.76)	68	14	18 (78.82)

The figures in parentheses indicate the inhibition of germination over the initial germination
FS = Final stand .

in moisture retaining capacity of gunny bag and bomboo basket, the total curtailation of moisture entry by iron bin and high moisture absorbing and retaining capacity of earthen pot could account for such results.

The storage of seeds in earthen pot had a negative effect on the overall rate of germination. The impact of storage on seed was slightly better in bamboo basket followed by gunny bag. Seeds stored in iron bin exhibited a very low level of mortality. It is apparent from the findings that the germinalibility of the seeds in all the containers showed a reduction with the increase in storage period. Similar pattern was observed for the final stand (%) in all the cases investigated.

On performing the F-test to test the significance of variance of percentage infection in *Phaseolus vulgaris* Linn. seeds stored in different containers, insignificant variation in percentage fungal infection in *Phaseolus vulgaris* Linn. seeds was observed between gunny bag and bamboo basket, gunny bag and iron bin, bamboo basket and iron bin and between bamboo basket and earthen pot, while significant variation was observed between gunny bag and earthen pot and between earthen pot and iron bin at 0.05 level of significance (Table 3)

Table 3. Analysis of variance (ANOVA) to test the significance of variance of percentage fungal infection in *Phaseolus vulgaris* Linn. seeds stored in different containers.

Storage Containers (IN PAIRS)	Calculated F Value
Gunny bag and bamboo basket	0.53434(NS)
Gunny bag and earthen pot	0.82609*
Gunny bag and iron bin	0.17618(NS)
Bamboo basket and earthen pot	2.01628(NS)
Bamboo basket and iron bin	1.25942(NS)
Earthen pot and iron bin	6.61933*

NS = Not significant
*= significant at 0.05 probability level.

From the present investigation, it can be concluded that the condition of the seeds stored in containers viz . earthen pot, bamboo basket, gunny bag and iron bin revealed that none of the storage containers were totally effective or suitable for such purpose for none of these containers were free from fungal infestation. Among the storage containers, the iron bin was the best suited as compared to the other containers in terms of seed viability and less moisture retaining capacity. In contrast, the earthen

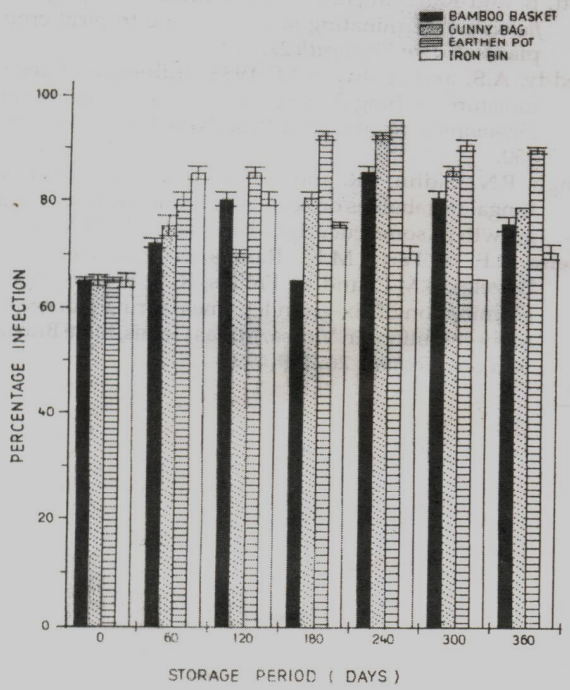

Fig. 1. Percentage infection of *Phaseolus vulgaris* Linn. seeds stored in different containers.

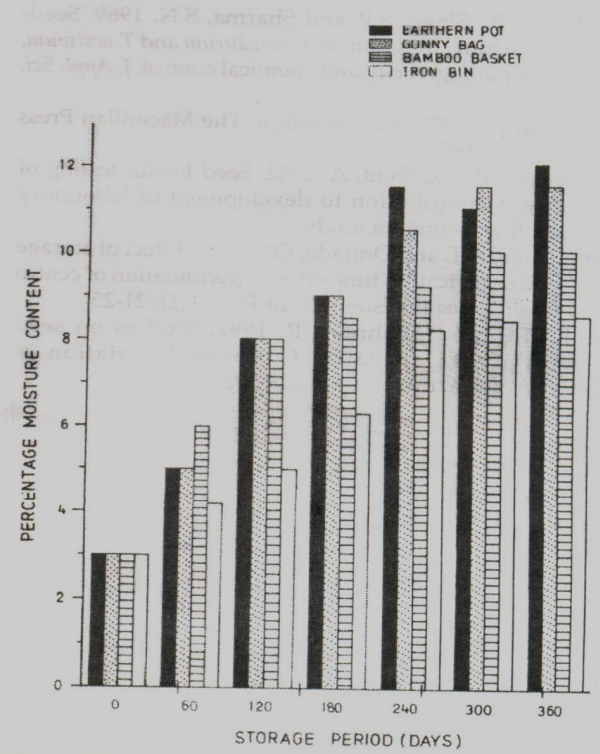

Fig. 2. The percentage moisture content of *Phaseolus vulgaris* Linn. seeds stored in different containers.

pot proved to be the least effective storage material due to the presence of a large amount of moisture inside and thus providing an ambient condition for the development of large number of fungal colonies in the seeds. It was further observed that with an increase in the storage period, the viability or germinability of the seeds also showed a declining trend. The storage fungi may induce a decrease in germination percentage. The activity of the fungi depends on the physical condition, vitality and moisture content of the seeds, temperature and relative humidity of the storage atmosphere.

ACKNOWLEDGEMENTS

One of the authors, Mr A. Bhattacharjee is thankful to the Head, department of Botany for providing laboratory facilities.

REFERENCES

Aulakh, K.S., Grewas, K.K. and Goul, R.K. 1976. Detection of seed-borne fungi of maize and their role in causing seed rot and seedling infections. *Indian Phytopath* 29 : 241-245.

Begnami, C.N. and Cortelazzo, A.L. 1996. Cellular alterations during accelerated aging of French bean seeds. *Seed Sci and Technol* 24 : 295-303.

Christensen, C.M. and Kaufman, H.H. 1965.Deterioration of stored grains by fungi *Ann. Rev. Phytopath* 3 : 69-84.

Christensen C.M. and Kaufman H.H. 1969. "Grain storage" Univ. of Minnesota Press, Mineapolis. 7-157.

Christensen, C.M. and Lopez, L.C. 1963. Pathology of stored seeds. *Proc. Int. Seed Test Ass.* 28 : 701-711.

Christensen, C.M. and Mirocha, C.J. 1976. Relation of relative himidity to the invasion of rough rice by *Aspergillus parasiticus. Phytopatho* 66: 204-205.

CIAT. 1992. Trends in CIAT commodities. Working document III CIAT, Cali Colombia.

ISTA. 1966. International Rules for Seed Testing *Proc. Int Seed Test. Assoc.* 31 : 1-52.

ISTA. 1976. International Rules for Seed Testing, Annexes, 1976. *Seed Sci Technol* 4: 3-49, 50-177.

Kumar, S.M. and Nema, K.G. 1973. Role of fungi associated with wheat seeds. *Jawaharlal Nehru Krishi Vishwavidyalaya Res.J.* 7(4): 277-279.

Laing, D.R., Jones, P.J. and Davis, J.H.C. 1984. Common bean *(Phaseolus vulgaris* L.), in *P.R.Goldsworthy and N.M. Fisher (eds).* The Physiology of tropical crops. Wiley, New York. 305-351.

Limonard, T. 1966. Amodified blotter test for seed health *Neth. J. Pl. Path.* 72(6): 319-321