STUDIES ON SEED MYCOFLORA OF CERTAIN LEGUMES

M. Phil. Dissertation

BY

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Man's struggle from the time of his existence has been for the quest of food for his hunger. He has tried to increase the food supply by evolving new high-yielding varieties and controlling various hazards. Most of the world's food supply is drawn largely from two families, i.e., Graminae and Leguminosae.

Legumes occupy an important position in man's major part of the diet. The seeds of legumes are rich (25-40%) in protein which are essential in a balanced human diet. Seeds after harvest are exposed to a variety of fungi designated as post harvest fungi and during storage as storage fungi. These fungi result in their deterioration ranging from decrease in germinability to complete spoilage. Such seeds harbour a great variety of microflora specially fungi. A large no. of species of it are already known to be actively engaged in biodeterioration of various seeds and grains.

Most of the storage fungi have been found to belong to two genera, Aspergillus and Penicillium. These fungi flourish under the same environment which is conducive to other fungi, viz., nutrition, water, a favourable temperature, etc. The rate at which these fungi develop on any given lot of seeds depends greatly on the history and condition of the storage, the degree of invasion inside the
seeds, the condition of seeds, i.e., broken or entire seeds, etc.

Considerable work has been done on seed mycoflora during storage, both in India and elsewhere and the literature has been reviewed from time to time (Tuite, 1959; Malone and Muskett, 1964; Christensen, 1970) etc. At least twenty species of fungi have been isolated from seeds of soybean while Alternaria species have been recorded from more than 200 species of plants effecting the seed quality of those plants. Christensen and Kaufman (1965) observed that these field fungi may discolor seeds, cause death of the ovules, shrivelling of the seeds or kernels, cause weakening or death of the embryos and result in the development of compounds toxic to man and other animals. These toxic compounds have been designated as mycotoxins. Because of the possible importance of these mycotoxins in the health of humans and of domestic animals they are now undergoing fairly extensive and intensive investigations all over the world. In some cases in addition to the above symptoms the nutritive value is also reduced due to the infection of fungi.

The earliest record of the study of seed-borne fungi show their importance from the very beginning. In Switzerland as early as 1816 a law was promulgated regarding seed
testing. But in the real sense the seed testing got importance during the later part of the nineteenth century when the association of fungus with germinating seeds was observed by workers engaged in seed testing. According to Malone and Muskett (1964), Bessey (1886) published the results of germination tests with notes on the fungi recorded. Smith (1901) gave illustrated notes on fungi detected on germinating farm seeds. In 1905 the first meeting of international analysts was held in Wien. Later a society of official seed analysts of North America was formed in 1908. Leningard plant protection station came into existence in 1920 and Dorogin started working on seed-borne fungi of crops in Russia. The first seed testing station was set up at Wageningen in The Netherlands. In 1923 Doyer published a handbook consisting of methods of detection of seed-borne organisms, listing and classification of microorganisms and their control measures. Important studies have been made on different aspects of seed pathology by Russell and Ledingham (1941), Gordon (1944), MacWhorter and Miller (1944), Leukel and Martin (1950), Tai and Singh (1976), Triffins and Amin (1977) etc.

Histopathological studies have been carried out to locate the site of seed infection and its role in transmission of the infection during the subsequent years. In some cases the fungi were held mechanically and protected
in the crevices of the seeds.

In an attempt to improve the germination of seeds harbouring fungi, seed treatment has been practiced for over three centuries. In about 1660 brine was accidentally discovered to be effective against the bunt of wheat. At the end of the eighteenth century Abbé Tessier in France tested many chemical compounds to improve wheat seed without affecting agricultural practice (Woolman and Humphrey, 1924). Recently antibiotics have been shown to be effective against pathogenic fungi and particularly bacteria. Besides, studies have also been made to improve storage conditions to allow minimum fungi to spoil. Air tight and cold storages or storages at moisture contents too low for fungi to grow have given good results thus prolonging the viability and maintaining quality in most agricultural seeds.

In view of the fact that legumes are next in importance to cereals as source of human food there is need to increase their availability to human diet. This can be done by increasing the acreage and controlling disease hazards. The literature on the seed mycoflora of legumes in general and pigeon pea and lentil in particular shows that no systematic work has been done on these crops on these aspects. Hence it is considered desirable to study the seed mycoflora in detail and to work out probable control methods. Since
chemicals are expensive and hazardous attempts will also be directed to explore the possibility of using indigenous plant by-products for controlling seed mycoflora. Besides, the effect of culture filtrates of certain fungi on other pathogenic fungi will also be studied.

In nature when seeds are sown it is not one fungus in isolation which is affecting the seed germination but always together, therefore, the culture filtrates will be mixed together and will be tested for its effect on seed germination.

*Trichoderma viridae* is a soil inhabitant and has the property of suppressing the activity of certain pathogenic fungi. Recently Singh (1984) showed that in seeds coated with culture filtrate of *T. viridae* penetration of root-knot nematode larvae and development of galls was reduced. Since no work has been carried out to determine the effects of this fungus on seed mycoflora it is considered feasible to test it.

**Plan of work**

(1) Inspection of seeds of different varieties of pigeon pea and lentil for seed health with naked eyes and under stereo-binoculars.

(2) Study of external seed mycoflora of pigeon pea.
and lentil.

(3) Study of internal seed mycoflora of pigeon pea and lentil.

(4) Study of certain important seed-borne fungi of pigeon pea and lentil for pathogenicity.

(5) Study on the efficacy of different fungicides against seed mycoflora of pigeon pea and lentil.

(6) Study the effect of different fungicides on nodulation in pigeon pea and lentil.

(7) Effect of certain plant products for controlling seed-borne mycoflora of pigeon pea and lentil.

(8) Effect of culture filtrates of certain fungi on seed mycoflora and germination of seeds.
REVIEW
of
LITERATURE
General seed pathology

Most of the work on seed pathology has been confined to testing of fungi and controlling them with chemicals. Wu et al. (1979) observed association of hypha and conidia of *Alternaria brassicicola* with ungerminated seeds of cauliflower. Kushi and Khare (1979) while working on seed-borne fungi of sesame (*Sesamum indicum*) and their significance found that *Macrophomina phaseolina* was most abundant followed by *Corynespora cassicola* and *Alternaria sesami*. Wu (1979) during the survey of seed-borne fungi of vegetables such as onion, radish, cabbage, chinese cabbage and black salsify (*Scorzonera hisponica*) observed that seeds of *S. hisponica* were extremely sensitive to 1% sodium hypochlorite treatment. Koch (1979) made field testing of different fungicides for over ten years and found that Thiram gave good protection against *Fleospora betae*. Diaconu (1979) observed that systemic fungicides such as *Tidomil 25 W.P* and *Curzate* proved very effective agents for *Pythium* spp. and *Rhizoctonia solani* on cucumber. Tiradhana et al. (1979) reported that Metalaxyl (1 gm a.i./kg seed) gave complete protection to grains of maize in the field against *Sclerospora sorghii*. Gupta (1979) controlled damping-off of guava caused by *Rhizoctonia solani* with Bavistin and Brassicol at 3 and 5 gm/kg seed respectively. Sivaram (1980) treated seeds of crucifer with hot water for prolonging the storage of seeds.
Vannacci and Gambogi (1980) made a detailed study on Fusarium solani f. sp. cucurbitaceae race - 1 infecting seeds of Cucurbita pepo L. pertaining to identification of the pathogen, effect of cultural condition on the course of infection and control. Seed dressings with Benomyl, Thiram or Benomyl plus Thiram gave effective control of the disease. Iliescu et al. (1980) obtained satisfactory control of sunflower disease by chemical treatment. Ferrer et al. (1980) controlled chemically Drechslera oryzae and Trichoconis pedwickii transmitted by rice seeds by applying Sisthane or Chlorothalonil in the former and by different fungicides in the latter. Falina (1981) reported that smuts of cereal crops (Ustilago and Tilletia spp.) caused severe losses in USSR which could be controlled by seed treatment with Fundazol. Sharma et al. (1981) obtained control of Peronosclerospore philippinensis by treating maize seeds with Metalaxyl.

Sarman and Frasad (1981) observed that seed treatment by Bavistin reduced death due to root rot and stem rot caused by Macrophomina phaseolina on jute while Carbendazim, Benlate and Dithane M-45 increased the yield of fibre. Huang and Sun (1962) reported that Benomyl seed dip controlled tomato wilt caused by Fusarium oxysporum f. sp. lycopersici. Chatrath and Gupta (1982) observed incidence of Ustilago nuda less when the seeds had been treated with Carboxin and then washed with water/acetone. Bateman (1983) found
incomplete control of seed-borne *F. nivale* in wheat and barley by seed treatment with Phenylmercuryacetate which appeared to be due to infection being deeply sited. Muthuswamy *et al.* (1983) while working with nine different fungicides obtained improved germination of chilli. In the studies of seed mycoflora of six varieties of *Eleusine coracana* L. (ragi) *Drechslera nodulosa* predominated followed by others (Dutta and Jha, 1983) and seed treatment with Bavistin plus TMTD (Thiram) eradicated the pathogens and enhanced germination. Kumar *et al.* (1983) established seed-borne nature and infectivity of *Fusarium solani* — the causal agent of wilt and fruit rot of brinjal (*Solanum melongena* L.). Osinska *et al.* (1985) obtained good control of sugarbeet black leg with Thiram and Tachigaren. Rattori *et al.* (1985) observed *Cylindrocladium clavatum* — the cause of seedling disease of *Eucalyptus* hybrid on 1° of seed. Strandberg (1983) observed heavy infection and colonization of inflorescence and mericarp of carrot by *Alternaria dauci*. Shothri *et al.* (1985) isolated 21 seed-borne pathogenic fungi from *Gaillardia* which could be controlled by seed treatment with Dithane M-45 (0.3%) and Aurofungin (0.01%). Srivastava and Gupta (1983) found that seed-borne fungi caused heavy infection on *Zinnia* resulting in seed rot and death of seedlings. These were however, controlled with Dithane M-45 (Mancozeb). Seed-borne pathogenicity of *Alternaria tagetica*
on Tagetes has been established by Hotchkiss and Baxter (1983). Venkatasubaiah and Safeulla (1983) observed low percentage of collar rot by Rhizoctonia solani by treating with Thiophanatemethyl. Sinha and Singh (1983) observed sugarcane seed decay due to Curvularia spp. and Drechslera spp. in the field which could be controlled by seed treat­ment with fungicides. Agarwal et al. (1983) obtained control of 19 fungi on stored ajwain seeds (Trachysperma ammi) caus­ing rot by Captan. Sachidanantham et al. (1983) studied the effect of seed treatment with fungicides on the viability of some oil seeds during storage. Dithane M-45 (Mancozeb) proved to be the best. Mittal (1983) while isolating 26 species of fungi from the seeds of some forest trees found nine causing deep-seated internal infections. Seed treatment with AH 2161 was found to be most effective. Rhizoctonia solani causing damping-off of citrus seedlings was effect­ively controlled by seed treatment with Bavistin (Carbendazim) and soil drenching with Captan in pot tests with rough lemon (Citrus jambhiri) Rao (1984).

Seed pathology of legumes

Asnworth et al. (1964) established that seeding blight of peanut is caused by seed-borne Aspergillus niger. Kennedy (1964) examined the effect of moisture content on mold invasion and seed viability of stored soybeans. Ujevic et al.
(1965) observed that *E. parasitica* (? *E. cinerea*) and *F. oxysporum* were dangerous parasites of lentil and were seed-borne. Frezzi (1967) worked out the factors affecting quality of seeds as influenced by fungi. Welty and Cooper (1968) observed that prevalence of storage fungi in groundnut is influenced by storage temperature and moisture content. Chohan and Gupta (1968) discovered a new seed-borne disease of groundnut caused by *Aspergillus flavus* known as afla rot disease. Bell (1969) while comparing the pathogenicity of fungi to sound and damaged peanut seeds in known fungal culture at four temperatures found that pathogenicity is dependent upon temperature. Interesting results have been obtained on seed mycoflora of fresh and stored groundnut kernels (Joffe, 1969), soybean (*Glycine max*) (Mishra et al. 1969) and cowpea (Sackston, 1969). Kraft (1969) discovered chickpea -- a new host of *Fusarium solani* f sp. *pisi*. Lisker et al. (1970) studied penetration of *Aspergillus flavus* and some other fungi into pots of various peanut varieties. McDonald (1970) observed that fungal infections of groundnut was different when studied before harvest, after maturity and during drying of the fruit. McGee and Christensen (1970) studied the storage fungi and the resultant fatty acid content in seeds of soybean and sunflower. They found that the fatty acid content was reduced due to storage fungi. Halfon-Meiri (1970) observed that seed-borne...
Ilyas et al. (1975) were able to locate the position of the *Diaporthe phaseolorum* var. *sojae* and *Cercospora kikuchii* in infected soybean seeds. Winter and Jauch (1975) reported *Fusarium equiseti* on the fodder soybean seeds. Rosca (1976) investigated the distribution and intensity of infection of soybean seeds by *Peronospora manshurica*. Tandon (1977) reported *Achaetomium* as a new seed-borne pathogen of *Phaseolus aureus*. Saxena and Sinha (1977) reported certain isolates of *Colletotrichum truncatum* from seeds of mungbean and urid-bean. Shukla and Bhargava (1977) reported *Fusarium solani* from seeds of different oil crops and pulses. Sinha and Prasad (1977) and Shukla and Bhargava (1978) observed deterioration of 'arhar' seeds by *Aspergillus flavus*. 'Uridha and Takir (1978) observed that *Macrophomina phaseolina* and *Sclerotium rolfsii* caused failure in germination of seeds of groundnut and that they were seed transmitted. Kellerly and Sinclair (1976) observed losses in seed quality due to *Phomopsis sojae*. Rodríguez-Marcano and Sinclair (1976) reported fruiting bodies of *Colletotrichum dematium* var. *truncata* and *Phomopsis sojae* on soybean seeds. Patnaik et al. (1978) detected *Peronospora manshurica* in seeds of soybean. Kmetz et al. (1978) pointed out that *Diaporthe phaseolorum* var *sojae* and *D. phaseolorum* var *caulivora* caused seed decay of soybean. The frequency of occurrence of *Phomopsis* was higher. Dhingra (1978) found *Fusarium*
semitectum, Phomopsis spp. and newly reported Trichotheicum roseum associated with reduced seed quality of dry snapbean. Agrawal and Jain (1978) observed Drechslera subspendorfii Mouchacca on seeds of Phaseolus aconitifolius collected in Rajasthan. Saxena and Sinha (1978) observed Ascotricha chartarum, Colletotrichum truncatum and Fusarium oxysporum infecting seeds of Vigna radiata var. radiata. Kellock et al. (1978) pointed out seed-borne Fusarium spp. on subterranean clover and other pasture legumes. Lenne and Sonoda (1978) found that Rhizopus stolonifer was the commonest fungus associated with Stylosanthes hamata L. seed harvested at Fort Pierce. Thompson et al. (1979) found the association of Sclerotinia sclerotiorum on soybean seeds for the first time in South Africa. Girard (1979) detected Colletotrichum dematium var. truncata and Diaporthe phaseolorum var. sojae on soybean seeds from Senegal. Dhingra et al. (1979) suggested that late planting of early maturing cvs. of soybean for production of healthy seeds of soybean and controlling T. c. infection Phomopsis sojae and Fusarium semitectum. Moravčík (1979) studied Ascochyta pisi and Mycosphaerella pinodes causing anthracnose mottle disease of pea seeds highly suppressive for germination. Scholefield and Griffin (1979) discovered Vigna radiata as new host for Macrophomina phaseolina from U.K. Rhizoctonia solani was found to be a seed-borne pathogen in Malaysia (Van Zainum and Yap, 1979).
Kolodiichuk (1979) isolated *Fusarium oxysporum* and *F. culmorum* from seeds of red clover reducing their germination by 13%. Yeh and Sinclair (1980) reported *Chaetomium cupreum* from soybean seeds. The ethylether soluble fractions of the culture filtrate of the fungus inhibited the growth of many other fungi and delayed germination of soybean seeds. Kovics (1980) identified *Phytophthora megasperma var. sojae* causing *Phytophthora* rot in soybean in Hungary. Mycoflora of a cultivar of *Dolichus lablab* predominantly constituted of *Trichothecium roseum*, *Alternaria* spp. and *Fusarium* spp. causing discoloured pods and shrunken discoloured seeds (Siddaramaiah, 1980). Kellock *et al.* (1980) observed seed-borne infection of subterranean clover by *Fusarium* spp. Pangtey and Sinha (1980) described *Colletotrichum capsici* and *Phoma medicaginis* on *Dolichus biflorus* (horse gram) seeds (both are new host records) causing leaf spot diseases. Ploper (1981) described *Thanatephorus cucumeris* causing web blight, a new disease, of *Phaseolus vulgaris*. Cardenas and Angleman (1981) showed the change in seed anatomy due to attack of *Colletotrichum lindemuthianum* on *Phaseolus vulgaris*. Sasaki (1982) examined the progress of disease caused by *Cercospora kikuchii* on soybean. Microscopic observations revealed lesion formation every two to seven days from the cotyledonary to harvest stage. According to Castro and Kimati (1981) seed infection by microorganisms
of soybean seed was unaffected by sprays of growth regulators. Dimitrijevic and Jurkovic (1982) observed *Phomopsis sojae* causing severe infection of soybean. Yehia *et al.* (1982) studied root rot disease of broad bean in Iraq, caused mainly by *Fusarium solani*. The infection reduced plant growth with weak root system. Hill and West (1982) observed penetration of fungal hyphae to soybean seeds through pores. Kulik and Yalilich (1982) found a relationship of the appearance of soybean seeds to seed-borne infection by *D. phaseolorum*. Lemos and Loch (1983) observed that high RH favoured the infection of *Cercospora kikuchii*. Garzonio and McGee (1983) found that seed crop residues are sources of inoculum for pod and stem blight of soybean. Sutton and Deverall (1983) observed that seeds of bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) are infected with ascospores of *Sclerotinia sclerotiorum*. Vesper *et al.* (1983) found that *Verticillium nigrascens* in soybean reduced the number of pods per plant and the weight of seeds. Singh *et al.* (1983) found that the pH of the water influenced the frequency of isolation of twelve fungi associated with soybean seeds. Tekrony *et al.* (1983) observed a relationship between weather and soybean seed infection by *Phomopsis* spp. Khan *et al.* (1983) reported *Ascochyta fabae* causing blight on faba bean in Pakistan. Michail *et al.* (1983) observed the infected faba bean seeds and plant debris act as main
source of *Ascochyta* leaf and pod spots. Gomes and Dhingra (1983) pointed out *Alternaria alternata*, a serious pathogen of white coloured snapbean, *Phaseolus vulgaris* seeds caused pod flecking, stem and petiole necrosis and seed discolouration. Rashid et al. (1983) isolated a large number of seed-borne fungi from mung and many of them were found to be pathogenic. Similarly Zaidi and Prakash (1985) isolated large number of seed-borne fungi from cowpea seeds and a few of them caused disease and deterioration of seeds. Tekrony et al. (1984) found that the date of harvest, maturity and soybean seed quality affected seed mycoflora and frequency of *Phomopsis* spp. on seeds. Maheshwari et al. (1984) observed that infection of *Aspergillus* spp. and * Fusarium moniliforme* influenced total sugar of lobia seeds stored at different RH and temperature. Jossen and Morrell (1984) studied the effect of *Ascochyta* blight on quality loss of lentil at harvest. Trijhuwan and Sinclair (1985) pointed out changes in histopathology of soybean seeds infected with *Cercospora sojina*. 
Control measures

Chemical control: For controlling seed-borne pathogens chemicals have been mostly recommended. Vidhyasekaran and Arjunan (1976) tested large number of fungicides for the control of storage fungi in *Phaseolus mungo* (black gram) and found Thiram and Captan increased yields. Control of Anthracnose of chickpea caused by *Mycosphaerella rabiei* has been achieved by chemicals (Senyurek et al., 1977; Verma and Vyas, 1977). Haware et al. (1978) observed complete control of *Fusarium oxysporum* f. sp. *ciceri* in chickpea seeds by seed treatment with Benlate T (Benomyl plus Thiram). Vyas and Nene (1978) observed that the persistence of Thiram on gram is influenced by moisture. Shirsat and Kale (1979) observed that seed treatment of chickpea with Iprodione, Thiophanate-methyl, Quintozene and Mancozeb fungicides not only increased seedling vigour but also improved germination. Reddy (1980) found that Calixin M (Tridemorph) alone or with Benomyl gave complete control of *Ascochyta rabiei* in chickpea seeds. Viswakarma and Basucauchinary (1982) used systemic and non-systemic fungicides for the seed treatment to control root diseases of gram. Kakralia and Mehrotra (1982) found that treatment of *Cicer arietinum* seeds at the highest rate 20° of Brassicol (FCWB) gave an adverse effect by causing stunting and fusion of leaflets in the developing seedlings.
Bhatti et al. (1983) found that none of the seven test fungicides eradicated *A. rabiei* from infested seeds of *Cicer arietinum*. Matrajan and Bhagyaraj (1984) studied the effects of fumigation on microflora and the viability of black gram and field bean seeds and found an adverse effect in the beginning. Gurdip Singh and Bedi (1980) obtained good control of foot rot of gram caused by *Operculella padwickii* using fungicides. Petkar et al. (1977) found Benlate most effective as a seed dressing to control collar rot of double bean (*Phaseolus lunatus*) caused by *Macrophomina phaseolina*. Grover and Chopra (1977) provided evidence that seed, soil and foliage treatment with Carboxin and Oxycarboxin gave good control of *Rhizoctonia* spp. and other fungi. Root rot of *Phaseolus mungo* caused by *Rhizoctonia solani* was controlled by Carboxin seed treatment. Oxycarboxin was effective only against *Macrophomina phaseolina*. Dhingra and Maffia (1978) found that acetone could be used as a fungicide carrier in dormant snap bean seeds without harming the seeds. Charbanda and Bernier (1979) studied the effectiveness of seed and foliage application of fungicides to control *Ascochyta blight* of faba beans using systemic and non-systemic fungicides both in lab and field. In the lab all seed treatments containing Benomyl or Thiabendazole effectively controlled seed-borne infection of *A. fabae* but in field none of the seed treatments significantly reduced
seedling infection but foliar sprays of Chlorothalonil gave the most promising results and was also evaluated on other cultivars. Seed treatments where the disease was already established in a crop Chlorothalonil was most effective as foliar spray in reducing infection. Clarke and Kellock (1979) found that the addition of either Benomyl or Thiabendazole at 500 ppm to the Rhizobium inoculum was an efficient method of dressing lupin seeds to eradicate Phomopsis sp., if sown in areas without any previous history of lupinosis (P. leptostromiformis). Jalali and Grover (1979) observed that Chloroneb was effective against R. solani which moved systemically to growing parts after absorption through germinating seeds of Vigna mungo. Raynal (1980) found that damping-off of lucerne caused by Pythium ultimum was controlled by seed treatment with CGA 48988 (Metalaxyl) in low doses. Dhingra and Muchovej (1980) and Muchovej and Dhingra (1980) used successfully Dichloromethane, Trichloromethane, Carbon tetrachloride, acetone, benzene and ethanol as solvent for systemic fungicides used for bean seed treatments. Cladiran and Okusanya (1980) reported that most of the fungicides effectively suppressed the pathogen associated with basal stem rots (Pythium aphanidermatum, Sclerotium rolfsii, Macrophomina phaseolina and Botryodiplodia theobromae) of cowpea in Nigeria. Sindhan and Bose (1981) reported that Benlate as seed dressing and foliar spray as well, effectively controlled anthracnose of french bean caused
by *Colletotrichum lindemuthianum* followed by Bavistin and Vitavax. Kore and Solanke (1981) obtained considerable reduction of seed mycoflora and increase in the longevity of seeds of walbean (*Dolichos lablab*) when treated with fungicides. Karwasra and Mohinder (1982) found that seed treatment with Agrosan GN, Captan, Dithane Z-78 (Zineb) and Dithane M-45 (Mancozeb) were most effective in controlling seed mycoflora of clusterbean. Sharma and Sohi (1982) while applying different fungicides against *Rhizoctonia solani* causing root rot of frenchbean (*Phaseolus vulgaris*) found best protection by Benlate (Benomyl) and NF-48. Kore and Solanke (1982) obtained highest percentage germination of frenchbean seeds treated with Agrosan GN (phenylmercury-acetate and ethylmercurychloride), Vitavax (Carboxil) and Carbendazim. Locke *et al.* (1983) applied Metalaxyl to snapbean seeds to reduce Pythium blight. Dang *et al.* (1983) successfully controlled pearl millet downy mildew caused by *Sclerospora graminicola* with systemic fungicides.

observed that in dry lentil seed dressing Bavistin was most effective of the eleven fungicides tested followed by Thiram and Captan in improving germination, plant stand and yield. Makram and Sidky (1968) obtained good control of Fusarium spp. and Rhizoctonia solani causing root rot and damping-off of peas by Spergan and Hercules 3944 and an increase in yield. Gangopadhyay and Kapoor (1976) found that Fusarium oxysporum f. sp. pisi was controlled by soaking pea seeds in a 1:1 suspension of Captafol (0.1%) and Captan (0.2%) for 30 min at 30°C. Utikar et al. (1978) applied successfully four systemic, four non-systemic and an antibiotic against F. oxysporum f. sp. pisi causing fusarium wilt in peas. Seed treatment with Bavistin (Carbendazim) and Benlate (Benomyl) gave complete control. Batalova and Zinov'ev (1978) found that fungicide seed protectants were safe for nodule bacteria. Of the 21 fungicide seed protectants they tested, 13 were not bactericidal to nodule bacteria of V. In Hewett (1979) reported hypochlorite pretreatment in the agar plate test for Ascocytta spp. and found that increasing the pretreatment from 30 s to 40 min did not effect the results of tests for A. pisi on peas and A. fabae on beans. Locke et al. (1979) found that the application of the systemic fungicide Metalaxyl (CGA - 48988) direct or by acetone infusion significantly reduced seed rot of peas caused by Pythium ultimum. Aphanomyces euteiches causing root rot of pea has been controlled by Tachigaren (Kotova and Tsvetkova,
1979; Tsvetkova and Guseva, 1980) but Bavistin gave good control of *Fusarium oxysporum* f. sp. *pisum* causing pea wilt (Shukla et al., 1979).

Lai and Mathur (1967) and Bell (1968) observed the relationship of peanut seed treatment with fungicides to seed mycoflora and germination. In general blends of two fungicides were superior to single fungicides. Frank (1969) found that three parts Captan plus one part Quintozene at 3 gm per kilogram seed gave satisfactory control of *Aspergillus niger* and *Rhizopus* spp. causing rots of groundnut. Mathur and Sharma (1971) found that of the 11 fungicides tested in the field Cereson at 3 gm per kilogram improved germination of groundnut seeds. Kadian and Suryanarayana (1971) found that 0.2% Cereson wet and 0.2% Agallol-3 used as seed dips eliminated almost all seed-borne fungi on groundnut kernel. Of the dust tested Thiram at 1:300 and Agrosan GN at 1:400 gave the best control. Agnihotri and Sharma (1972) obtained highest control of *Aspergillus niger* causing collar rot of groundnut (*Arachis hypogea* L.) with Fertix 85, seed-dresser 6335 and seed dresser 6334 (upto 100%) the former 6335 being phytotoxic at higher doses. Mercer and Kisyombe (1978) while studying the fungal flora of groundnut kernel in Malawi found that in field trials seed dressing fungicides had little effect on yield and the practice was thus not generally recommended. Lal and Jayamma (1978) evaluated
seed dressing fungicide mixtures against *Aspergillus niger*, the incitant of collar rot of groundnut. Mixtures of Captan plus Thiram was found superior to other mixtures of fungicides. Siddaramaiah *et al.* (1979) obtained reduction in both pre and post emergence death due to crown rot of groundnut caused by *Aspergillus niger* by Carbendazim. Sharma and Bhowmik (1982) during their studies on the efficacy of twelve seed dressing fungicides against pre and post emergence seedling rot of groundnut caused by *Macrothomina phaseolina* found that Bavistin (Carbendazim) was the most effective.

Chemical control of pathogens on soybean seeds have been obtained by Anahosur *et al.* (1973), Carvalho *et al.* (1980), Czawa *et al.* (1980). Thiram and Carborin plus Thiram have been used as seed treatment against *Fusarium sojae* and *Fusarium* spp. attacking soybean seeds (Nyedrow and Harman, 1980). Borelli *et al.* (1980) studied effect of fungicide treatment of soybean seeds at different vigour levels on seedling emergence and vigour. Miller and Roy (1982) observed that Benomyl reduced the colonization of soybean leaves, pods and seeds by fungi. Marzocca (1983) observed no harmful effect on nodulation when Thiabendazole was applied at 200 gm per kilogram seed simultaneously with inoculation with *Rhizoctonia japonicum* in pot trials in the glasshouse.
Dwivedi and Tandon (1975) studied the efficacy of certain fungicides for seed treatment of pigeonpea. *Fusarium udum*, a wilt pathogen of pigeonpea was newly reported as seed-borne, in the same publication. Chaudhuri and Ahmed (1977) obtained effective protection against *Macrophomina phaseolina* on pigeonpea by Campogran M at 1.5 gm a.i. per kilogram seed, Allisan (dicloran) at 2.5 gm and Busan 72 (TCH73) at 0.3 gm. Ellis et al. (1979) observed improvement in field emergence due to seed treatment with fungicides. Both internal and external seed-borne fungi on pigeonpea has been successfully controlled by Captan, Thiram and Benomyl. Captan and Thiram being effective against those fungi present on seed coat and Benomyl to those present near embryo (Ellis and Paschal, 1979). Kotwal et al. (1981) observed that systemic fungicide Acylon (idomil) and Metalaxyl was effective equally as seed and root dip.

**Physical control:** UV radiation has been used for controlling *Ascochyta (Mycosphaerella) pinodes* causing ascochyta of pea (Peresypkin et al., 1966; Fathal et al., 1981). Athow and Laviolette (1973) obtained good protection against fungi by enclosing soybean pods in pollinating bags. Sinha and Khare (1977) pointed out that seed-borne *Macrophomina phaseolina* and *Fusarium equiseti* could be
controlled by hot water treatment (at 46°C for 20 min). Echeverry et al. (1983) found that in vitro the fungus *Cercospora kikuchii* causing purple discolouration of soybean was inactivated by treating seeds with hot water at 49°C for 5 min. Hall and Taylor (1983) used aerated steam treatment for control of *Alternaria tenuis* on lobelia seeds successfully.

**Application of antibiotics in controlling disease:** Penicillin has been used on soybean seeds pretreated with dichloromethane (Royse et al., 1975) or with aqueous polyethylene glycol solution (Hepperly and Sinclair, 1977). Tsvetkov and Donev (1984) reported effective control of root rot of *Phaseolus vulgaris* by antibiotics. Semi-wet treatment of seeds with Fusomycin and Sucomyacin reduced infection and increased yield.

**Biological control:** Biological control is difficult to achieve with seed-borne fungi but some attempts have been made in this direction on the principle that these fungi ultimately are incorporated to soil through seeds.

Agrawal and Khare (1974) found that germinating seeds of lentil exuded an antifungal chemical which could eliminate certain fungi. Agrawal et al. (1978) used *Trichoderma harzianum* and *Bacillus subtilis* antagonistic against
Sclerotium rolfsii to control collar rot of lentil. Cultures were more effective when applied to seed rather than soil. Some kind of biological control has been obtained by Wu (1980, 1982), Chu and Wu (1981) and Yeh and Sinclair (1982). Lindels and Kaumedahl (1978) studied the factors affecting *Penicillium oxalicum* as seed protectant against seedling blight of pea. Harman *et al.* (1980) protected seeds and seedlings of radish and pea against *Pythium* spp. and *Rhzoctonia solani* using conidia of *Trichoderma hamatum* nearly as effectively as fungicides. Singh and Mehrotra (1980) observed a satisfactory control of *Rhzoctonia bataticola* on gram by coating seeds with *Facillus* and *Streptomyces* spp. Pandey *et al.* (1982) studied volatile fungitoxic activity of some higher plants with special reference to that of *Callistemon lanceolatus*. The oil had no damaging effect on seed germination, seedling growth, general health and morphology of pigeonpea plants. Prasad and Simlot (1984) controlled the wilt of pigeonpea caused by *Fusarium udum* by a preparation from tezmu (*Diospyros cordifolia*) fruit.
MATERIALS

and

METHODS
International rules for seed testing (Anonymous, 1966) will be followed in the present investigation.

Seed samples of different varieties of pigeon pea and lentil will be obtained from the Indian Agricultural Research Institute, New Delhi and agricultural universities of the country.

Seed health studies
(1) Visual inspection of seeds: About 400 seeds of each variety of pigeon pea and lentil will be examined with naked eyes for presence of impurities like plant debris, discolouration, shrivelness and reduced size of seeds, fruiting bodies of fungi like sclerotia, mechanical damage of seeds. Mechanically damaged or cracked seed will be incubated for study of seed mycoflora. Seeds will also be examined under the stereo-binocular for the associated fungal structures like hyphae, perithecia, acervuli, pycnidia, sporemass etc.

(2) Studies of external seed mycoflora: For the study of external seed mycoflora 400 seeds of each sample will be placed in oven-sterilized Petri dishes lined up by three folds of blotter paper moistened by sterilized distilled water. Ten seeds per plate will be transferred under aseptic conditions to avoid contamination. The plates will be incubated at temperatures 22-28°C for 7-10 days under 12h
light and 12h dark cycles. The light source, a 40W tube will be 40 cm over the plate containing the seeds. The fungi appearing on seeds will be isolated in pure culture and identified. The number of seeds germinated will be counted.

The frequency of fungi will be calculated as follows:

<table>
<thead>
<tr>
<th>Frequency of fungi</th>
<th>No. of seeds containing particular fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>----------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Total seeds used x 10^6</td>
</tr>
</tbody>
</table>

(3) Study of internal seed mycoflora: For the study of internal seed mycoflora seed samples of the varieties of pigeon pea and lentil used earlier will be treated with 0.1% solution of mercuric chloride as surface disinfectant and washed several times by distilled water to remove the chemical. The seeds will be placed aseptically over moist blotter papers in Petri dishes and incubated at temperature (22-28°C) for seven to ten days as pointed out above. The internal seed fungi emerging from the seed will be isolated in pure culture and identified. Frequency of fungi will be calculated as per formula given above. The percentage germination of seeds will be recorded.

(4) Efficacy of certain bio-products and fungicides against the seed mycoflora: The plant products like Calatropisolate, different part or extracts of Argemone mexicana, Ranunculus, Tagetes, Azadiracta indica,
Papaver somniferum, Chenopodium ambricoides, Euphorbia spp., Lantana spp., garlic, turmeric, etc. will be tested against seed mycoflora. Extracts will be made in sterile distilled water.

Weighed quantities of seeds will be kept in 250 ml Erlenmeyer flasks and the required quantities of fungicides Sicarol, Bavistin, Benlate, Brassicol or Fytolane will be mixed with them. Flasks will be shaken for 15 min. so as to get uniform distribution of fungicides on seeds surface. Treated seeds will be placed over moist blotter paper in Petri dishes and incubated for 7-10 days at 22-25°C. Observations will be recorded for frequency of fungi and percentage germination of seeds. Untreated seeds (control) will also be kept in Petri dishes.

(5) Effect of fungicides on root nodulation: Seeds treated with fungicides will be sown in pots. Rate of nodulation in plants treated with different fungicides will be recorded after 45 days of sowing. The nodulation in plants grown from untreated seeds will serve as control.

(6) Pathogenicity test of certain important fungi: For pathogenicity tests 100 surface sterilized healthy seeds rolled on an actively growing culture of different fungi will be placed over moist blotter paper in sterilized Petri dishes. Equal number of uninoculated seeds will also be
kept to serve as control. Germination percentage and symptoms productions if any will be observed upto 20 days. Simultaneously 30 days old plants will be inoculated with a suspension of mycelium and spores prepared by maceration of 15 days old culture. Symptoms will be observed after 20 days.

(7) Effect of culture filtrates of certain fungi on seed mycoflora and germination of seeds: Pathogenic and non-pathogenic fungi isolated from soil will be cultured in Richard's liquid medium, the recipe of which is given below.

- $\text{KNO}_3$ ............... 10.00 gm
- $\text{K}_2\text{HPO}_4$ ............ 5.00 gm
- $\text{MgSO}_4$ ............... 2.50 gm
- $\text{FeCl}_3$ ................. 0.02 gm
- $\text{C}_12\text{H}_22\text{O}_{11}$ ......... 50.00 gm
- Distilled water .. 1000 ml

Erlemeyer flasks of 150 ml containing the sterilized above medium will be inoculated with the fungi recovered from seeds under aseptic conditions and these flasks will be incubated at 25°C in the incubator. After 15 days the mycelium mat will be filtered and the culture filtrate will be used for treating the seeds. Similarly the seeds will be treated with the culture filtrate of *Trichoderma viridae*.

The treated seeds will be kept in sterilized Petri
dishes lined with moistened blotter paper. In each Petri dish ten seeds will be kept. The percentage germination of seeds after every 24 h will be noted. The fungi appearing during the course of germination will be isolated in pure culture and identified. Untreated seeds will be kept in Petri dishes in the same way to serve as control.

The seeds treated with culture filtrate of *Trichoderma viridae* will be stored in small vials at room temperature. Before storing in vials the culture filtrate will be diluted to $5 \times 10^{-1}$, $5 \times 10^{-2}$, $5 \times 10^{-3}$ and seeds will be treated with each of them separately. Such seeds will be stored at room temperature. After 1, 2, 3, 4 and 8 weeks of storage the seeds will be plated to determine the seed mycoflora. Untreated seeds will be stored for similar periods. The fungi appearing will be identified and their frequency will be determined.

Further, those fungi providing health to seeds will be tested in detail. The culture filtrate of such fungi will be mixed in twos in various combinations. The seeds will be treated with culture filtrates and stored as above. Then effect on germination on seeds and fungi developing will be determined.

The data so obtained will be subjected to statistical analysis as and when necessary.
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REFERENCES


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