

Pathogenic Variation in Seed-Borne Fungi of Pulses

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Abstract

Pulses under study for their seed-borne fungi were Green gram (*Vigna radiata L.*), Black gram (*Vigna mungo L.*), Chickpea (*Cicer arietinum L.*) and Pigeon pea (*Cajanus cajan L.*). Overall, seventeen fungi were isolated from the seeds of test pulses. Among all seed-borne fungi six were found to be more pathogenic to the test pulses causing maximum infection. This pathogenic action of the seed-borne fungi ultimately caused loss of quality and quantity of the test pulses.

Key words: Seed-borne fungi; Pathogenic

Introduction

Pulses are the second most important group of food plants belonging to the family Leguminosae. They form an important and indispensable part of our daily diet. It is important source of dietary proteins, essential amino acids and micronutrients such as calcium and iron. Therefore pulses are important source of protein and essential amino acids for major vegetarians. About 88 percent of proteins consumed in India are of vegetable origin, dependence on animal proteins being very less. Legumes are greatly used as food and are next to cereals. They are also used as green manures due to root nodules containing nitrogen fixing bacteria.

The pulses like Green gram (*Vigna radiata*), Black gram (*Vigna mungo L.*), Chick pea (*Cicer arietinum L.*) Pigeon pea (*Cajanus cajan L.*) etc are cultivated in Marathwada region of Maharashtra during kharif and rabbi seasons, either as sole or intercrops, under rain fed or irrigated conditions. India has distinction of being the world's single largest producer of pulses. The area under production of pulses in India is approximately 20-24 million hectares. The major pulse growing states are Rajasthan, Madhya Pradesh, Maharashtra

Uttar Pradesh, Orissa, Bihar, Haryana, Andhra Pradesh, Tamil Nadu, Panjab, West Bengal and Gujraht.

Green gram (*Vigna radiata L.*): Green gram (*Vigna radiata L.*) is an annual plant with herbaceous bushy appearance. It attains a height of 1-3 feet, being more or less erect. The axillary raceme inflorescence is with variously yellow colored flowers in cluster. The fruit is typically a slender pod, measuring 3-4 inches long and bearing small, slightly flattened, globular seeds. The seeds are usually green in color but the cotyledons are used as dal. The plant requires 25-35 inches rainfall. It is cultivated both as a Kharif as well as Rabi crop. The Kharif crop is sown around June or July and Rabi crop in September or October. Within three months, the plant is harvested. Seeds show 24 g protein, 56.7 g carbohydrate/100g of edible part of the seeds, thiamin (0.47mg), and riboflavin (0.27mg), iron (7.3mg). (Shakuntala Manay and M. Shadaksharaswamy, 1987).

Black gram (*Vigna mungo L.*): Black gram (*Vigna mungo L.*) is an herbaceous annual plant with spreading procumbent branches, commonly referred as 'wooly pyool' due to presence of brown hairs covering stem. Inflorescence is represented by a long stout,

hairy axis bearing a group of 5-6 yellow flowers. In India it is commonly grown as a kharif crop where rainfall is 30-35 inches. Usually cultivated in June -July and harvested within 3-4 months. Commonly cultivated in Madhya Pradesh, Uttar Pradesh, Punjab, Maharashtra, West Bengal, Andhra Pradesh and Karnataka. Black gram is important for its high phosphoric acid content. It contains 24g protein/100g of seeds and carbohydrates 59.6g/100 g of seeds show that it is nutritious pulse. It also has good amount of phosphorus (385mg) iron (10.2mg), thiamin (0.42 mg), riboflavin (0.20mg), niacin (2mg) and vitamin C (3mg) (Shakuntala Manay and M. Shadaksharaswamy, 1987).

Chick pea (*Cicer arietinum* L.): Chick pea (*Cicer arietinum* L.) is small much branched plant attaining height of about 2 feet. The leaves are pinnately compound the Papilionaceous flowers are solitary and the pods contains one or two seeds. It is cultivated in dry cool climate during Rabi season in the regions with low to moderate rainfall. It is cultivated as intercrop along with Jowar, Wheat, and Bajra etc during October-November. The crop is harvested after about 3-4 months in February – March. It is mainly cultivated in Uttar Pradesh, Punjab, Rajasthan, Madhya Pradesh, Bihar, Maharashtra, Andhra Pradesh, West Bengal, Tamil Nadu and Karnataka. The malic and oxalic acids from the leaves of Chick pea are useful in intestinal disorders. It contains protein 20.5g/ 100g of seeds and carbohydrates 59.6 g/ 100g of seeds with thiamin (0.30mg), riboflavin (0.15mg), niacin (2.9mg), vitamin C (3mg) and phosphorus (312 mg) (Shakuntala Manay and M. Shadaksharaswamy,1987).

Pigeon pea (*Cajanus cajan* L.): Pigeon pea (*Cajanus cajan* L.) is an annual shrub of about 6-7 feet. The inflorescence is a typical axillary raceme bearing Papilionaceous flowers. It is cultivated as a mixed crop with Kharif cereals in low rainfall areas. Sowing is done in June – July and harvested after 6-8 months, between January- February. It is commonly cultivated in Uttar Pradesh, Orissa, Rajasthan, Maharashtra, Bihar and Tamil Nadu. It contains protein 20.4 g/100 g of seeds and carbohydrates are 60.4 g/100 g of seeds suggesting that it is also good source of protein and carbohydrates, it also contain thiamin (0.45mg), niacin (2-9mg) and riboflavin (0.19mg). It has better quality of fiber (7g/ 100g of seeds). (Shakuntala Manay and M. Shadaksharaswamy, 1987).

Pulse crops are affected by different fungal pathogens as seed mycoflora which is harmful to seed health and seed content and ultimately to yield. Association of the fungi with the seeds has found

to be harmful to the seed health and seed content. Such seeds are found to be harmful both for agriculture and human consumption. Such infected seeds may carry many pathogens; as shown by studies in the case of leguminous crops (Saxena and Sinha, 1977; Saxena and Gupta, 1981; and Maheshwari et al. 1984).

The term seed-borne fungi is used both for qualitative as well as quantitative nature of fungi occurring on or in the seeds (Neergaard, 1977). Various reports pertaining to seed mycoflora and its effects on seeds have been presented in the present review of literature.

Pulses are attacked by different seed-borne fungi. Such reports are recorded by Suhag (1973) and isolated fungi from Chick pea like *Aspergillus flavus*, *A. niger*, *Cladosporium fulvum*, *Curvularia lunata*, *Fusarium oxysporum*, *Rhizoctonia bataticola*, *Rhizopus nigricans* and *Aspergillus nidulans*, *Curvularia lunata*, *Fusarium spp.* etc. from Green gram. Singh and Chouhan (1973) identified fungal species like *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Curvularia* and *Rhizopus* from *Trigonella* spp. Similar studies pertaining to different crops were done by Jain and Patel (1969), Singh et al. (1974), *Rati and Ramlingam* (1974), *Tripathi* (1974), *Subramaniam and Rao* (1976), *Siddiqui and Ashok* (1978) and Singh and Singh (1979). Singh and Chohan (1974) studied seed mycoflora of cowpea and found that, fungi like *Aspergillus*, *Rhizopus*, *Cocliobolus*, *Drechslera*, *Fusarium*, *Penicillium* etc. were associated with the seeds. Singh and Chohan (1976) isolated six fungi associated with Black gram like *Aspergillus niger*, *Fusarium equiseti*, *Curvularia lunata*, *Fusarium oxysporum*, *Penicillium crustosum* and *Phoma glomerata*. Similar pathogenic study of seeds of pulses has been carried out by Saxena and Sinha (1979). Neergaard (1977) stated that, in general pulses carry load of different seed-borne fungi very commonly. Sinha (1979) studied seed mycoflora of Green gram and Black gram, and identified fungi belonging to genera *Aspergillus*, *Aschotrichina*, *Botrytis*, *Curvularia*, *Chetomium*, *Colletotrichum*, *Cercospora*, *Drechslera*, *Fusarium*, *Helminthosporium*, *Nigrospora*, *Penicillium*, *Phoma*, *Pleospora* and *Trichothecium*. Similar seed mycoflora study was done by Sinha et al. (1980) identified some important seed-borne fungi of pulses which caused reduction in seed germination. Deo Gupta (1980) showed that, *Aspergillus*, *Penicillium*, *Alternaria*, *Chetomium*, *Curvularia*, *Drechslera*, *Cliocladium*, *Monilia* etc were present on seeds of Gram. Qudsia and Prakash (1981) isolated twenty different fungal species from lentil seeds and reported that these fungi caused reduction in seed germination and malformation in seeds.

Agrawal (1981) studied seed mycoflora of Green gram and found fungi like *Cercospora kikuchii*, *Colletotrichum truncatum*, *Colletotrichum lindemuthianum*, *Curvularia lunata*, *Helminthosporium tetramera*, *Fusarium equiseti*, *Fusarium moniliforme*, *Fusarium semitectum*, *Myrothecium* and *Phoma*, *Alternaria porri*, *Cephalosporium* spp., *Macrophomina phaseolina*, *Myrothecium roridum* and *Periconia* spp. And *Macrophomina phaseolina*, *Aspergillus niger*, *Colletotrichum truncatum*, *Curvularia lunata*, *Drechslera australiensis* and *Rhizopus* spp. were isolated from Black gram variety pant U-30. Similar studies in respect to different seeds were carried out by Vijaykumari and Karan (1981), Shrotri et al. (1983), Thakur and Prasad (1983), Ghewande et al. (1984), Maheshwari et al. (1984), Yadav and Agnihotri (1985). Pandey et al. (1988) reported 20 isolates of *Aspergillus flavus* and two isolates of *Aspergillus parasiticus* from pulses. Similar studies were done by Mahajan and More (1989), Deshpande and Kulkarni (1990), Kannaiyan and Sithanatham (1991) and Krish and Rao (1992). Shah et al. (1992) isolated total 17 fungal species from cowpea seeds, *Aspergillus flavus* being the most common fungus. Kroschel et al. (1996) reported thirteen fungal species from *Striga hermonthica* seeds in Northern Ghana. Lal and Singh (1997) studied seed mycoflora of Green gram and 25 fungal species were isolated from seeds of cultivar pant-2 and T44. The predominant fungi were *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *A. luchuensis*, *Alternaria* spp., *Penicillium* spp., *Cladosporium* spp. and *Curvularia* spp.

Materials and Methods

Collection of seed samples

The methods prescribed by Paul Neergaard (1977) have been adopted for the collection of seed samples. Seed samples of Green gram, Black gram, Chick pea and Pigeon pea were collected from field, market places from Nanded district of Maharashtra. A composite seed sample for each of the pulse crop was made by mixing the individual seed sample together, preserved in gunny bags at room temperature during the studies.

Detection of seed mycoflora

The seed-borne fungi of different pulses, different categories and stored seeds of pulses were detected by moist blotter (B) and agar (A) plate methods as recommended by ISTA (1966), De Tempe (1970), Neergaard (1977) and Agrawal (1981). The procedure of moist blotter (B) and agar (A) plate methods is described as below.

Moist blotter plate method

In moist blotter plate method; a pair of white blotter papers of 8.5 cm diameter was jointly soaked in sterile distilled water and placed in pre-sterilized borosil glass Petri-plates of 10 cm diameter. Ten seeds were placed at equal distance aseptically on the moist blotter paper. The plates were incubated at room temperature for ten days. On eleventh day the seeds were examined under microscope for the preliminary determination of seed mycoflora. The seed-borne fungi found on each and every seed were isolated and identified, brought into pure cultures and maintained on PDA (Potato Dextrose Agar) slants for further studies.

Agar plate method

In agar plate method; 25 ml of sterilized PDA medium of pH 5.6 was poured in pre-sterilized borosil glass Petri-plate of 10 cm diameter. The Petri-plates were allowed to cool at room temperature; then ten seeds of test pulses were placed at equidistance under aseptic condition. The plates were incubated at room temperature for ten days. On eleventh day the seeds were examined under microscope for the preliminary determination of seed mycoflora. The seed-borne fungi found on each and every seed were isolated and identified, brought into pure cultures and maintained on PDA (Potato Dextrose Agar) slants for further studies.

Results and Discussion

All the test pulses were infected by seventeen fungi. *Alternaria alternata*, *A. tenuis*, *Aspergillus carbonarius*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *Chaetomium globosum*, *Cladosporium* sp., *Colletotrichum truncatum*, *Curvularia lunata*, *Drechslera tetramera*, *Fusarium moniliforme*, *F. oxysporum*, *Macrophomina phaseolina*, *Penicillium* sp. and *Rhizopus stolonifer*. These fungi showed infestation on the test pulses in variable percentages, from zero to 90%. The most common and dominant seed-borne fungi were *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Drechslera tetramera*, *Fusarium moniliforme* and *Rhizopus stolonifer* showed pathogenicity in terms of percent incidence in between 55% to 90%. Among these fungi *Aspergillus flavus*, *A. fumigatus*, *A. niger* was found to be most pathogenic and virulent, as shown in table. The seed mycoflora adversely affect to the test pulses caused reduction in quality and quantity of pulses.

Green gram (*Vigna radiata* L.) showed, *Aspergillus flavus* with maximum percent incidence on agar (A70%, B 67%), followed by *Aspergillus fumigatus* (A 61%, B 25%) *Aspergillus niger* (A 60%, B

55%), *Rhizopus stolonifer* (A 60%, B 18%), *Drechslera tetramera* (A 55%, B 55%), *Fusarium moniliforme* (A 45%, B 20%) and *Alternaria tenuis* (A 42%, B 25%). *Chetomium globosum*, *Colletotrichum truncatum*, *Aspergillus carbonarius* and *Cladosporium* spp. showed no incidence on blotter and very less incidence on agar plates. Remaining fungi showed minimum incidence both on blotters and agar plates.

Black gram (*Vigna mungo* L.) showed, Fungi like, *Aspergillus niger* (A 90%, B 67%), *Aspergillus flavus* (A 80%, B 60%), *Aspergillus fumigatus* (A 77%, B 57%), *Drechslera tetramera* (A 70%, B 58%), *Rhizopus stolonifer* (A 62%, B 33%) and *Fusarium moniliforme* (A 55%, B 48%) were found to be dominant on the seeds. *Chetomium globosum*, *Colletotrichum truncatum* were absent on blotter plate, whereas *Curvularia lunata*, *Penicillium* sp. were absent on agar plates and minimum on blotter. *Cladosporium* sp., *Curvularia lunata*, *Penicillium* sp. were least on blotters; whereas *Chetomium globosum*, *Colletotrichum truncatum*, and *Alternaria alternata* were least on agar plates.

Chickpea (*Cicer arietinum* L.) showed, *Aspergillus flavus* (A 86%, B 67%), followed by *Aspergillus niger* (A 82%, B 70%), *Drechslera tetramera* (A 70%, B 55%) and *Aspergillus fumigatus* (A 63%, B 48%). Fungi like *Alternaria alternata*, *Aspergillus carbonarius*, *Chetomium globosum*, *Cladosporium* spp. were absent on blotter and showed least incidence on agar. Similarly *Colletotrichum truncatum* was absent on agar and *Rhizopus stolonifer*, *Alternaria tenuis*, *Penicillium* spp. and *Macrophomina phaseolina* were least on agar and blotter.

Pigeon pea (*Cajanus cajan* L.) showed dominance of *Aspergillus flavus* (A 90%, B 62%), followed by *Aspergillus fumigatus* (A 80%, B 72%), *Aspergillus niger* (A 78%, B 52%), *Drechslera tetramera* (A 75%, B 60%) and *Rhizopus stolonifer* (A 58%, B 32%). Fungi like *Aspergillus carbonarius* on blotter and *Macrophomina phaseolina* on agar plate were absent. *Aspergillus carbonarius* (A 2%), *Macrophomina phaseolina* (B 7%), *Alternaria alternata* (B 12%, A 6%), *Penicillium* spp. (B 11% and A 28%), *Aspergillus nidulans* (B 6% and A 20%) were minimum on agar and blotter respectively.

Similar results were recorded by Coutinho et al. (1999) studied seed mycoflora of *Phaseolus vulgaris* and found *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium* spp., *Fusarium oxysporum*, *Penicillium* spp. and *Rhizoctonia solani* as its mycoflora. Dixit (1999) isolated 163 strains of *Aspergillus flavus* from Green gram and Black

gram. Bodke (2000) studied different cereal crops and its mycoflora and found that, the fungal species *Alternaria tenuis*, *Aspergillus flavus*, *Curvularia lunata*, *Drechslera tetramera* and *Fusarium moniliforme* were predominant and these fungi caused reduction in seed germination and seedling emergence. Murthy et al. (2003) studied Cowpea, Horse gram, Black gram and Green gram seeds and found that, *Macrophomina phaseolina*, *Fusarium semitectum*, *Fusarium moniliforme* and *Fusarium solani* were associated with seeds. Kritzinger et al., (2003) studied Cowpea seed samples from south Africa and Benin and found that, *Fusarium equiseti*, *Fusarium chlamydosporum*, *Fusarium graminearum*, *Fusarium proliferatum*, *Fusarium sambucinum*, *Fusarium semitectum* and *Fusarium subglutinans* were common seed-borne fungi. Baird (2004) studied pod and seed mycoflora of soybean and found 765 fungal isolates. Gorfu and Sanghole (2005) identified 16 fungal species associated with *Pisum sativum* which included *Alternaria alternata*, *Ascochyta pinodes*, *A. niger* and *A. flavus*. Kiran Singh et al. (2005) isolated nine fungal species namely, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium moniliforme*, *Helmintosporium sativum*, *Mucor* spp., *Penicillium notatum* and *Rhizopus stolonifer* from seeds of Chick pea. Toory et al. (2005) studied seed mycoflora of Chick pea seeds and recorded the fungi like *Aspergillus niger*, *A. flavus*, *Rhizoctonia* spp. *Rhizopus* spp. *Fusarium* spp. *Alternaria* spp. *Curvularia* spp. and *Ascochyta* spp. Agrawal et al (2006) inspected samples of soybean seeds imported to India from 1978-2004 and detected 21 pathogens including *Perenospora manshurica* which is not present in India. Patil et al. (2012), reported sixteen fungi on Pigeon pea and eighteen fungi on Chickpea seeds most predominant were *Aspergillus flavus*, *A. niger*, *A. carbonarius*, *Fusarium oxysporum* etc. Roopam Parashar et.al. (2019) reported thirteen genera and twenty three species of seed-borne fungi from four pulses, namely *Chickpea*, *Mungbean*, *Pigeon pea* and *Lentil*. The seed mycoflora of test pulses consisted of *Aspergillus carbonarius*, *A. niger*, *A. fumigatus*, *A. flavus*, *Curvularia lunata* etc. These fungi caused adverse effects on pulse seeds.

Sr. No.	Seed mycoflora	Incidence of seed mycoflora (%)							
		Green gram		Black gram		Chickpea		Pigeon pea	
		A	B	A	B	A	B	A	B
1	<i>Alternaria alternata</i>	15	10	06	15	10	00	06	12
2	<i>Alternaria tenuis</i>	42	25	40	22	35	18	25	27
3	<i>Aspergillus carbonarius</i>	12	00	20	29	28	00	02	00
4	<i>Aspergillus flavus</i>	70	67	80	60	86	67	90	62
5	<i>Aspergillus fumigatus</i>	61	25	77	57	63	48	80	72
6	<i>Aspergillus nidulans</i>	25	28	28	24	16	21	20	06
7	<i>Aspergillus niger</i>	60	55	90	67	82	70	78	52
8	<i>Chetomium globosum</i>	03	00	01	00	06	00	00	00
9	<i>Cladosporium spp.</i>	20	00	06	02	10	00	00	00
10	<i>Colletotrichum truncatum</i>	05	00	02	00	00	02	00	00
11	<i>Curvularia lunata</i>	23	12	00	05	27	35	36	31
12	<i>Drechslera tetramera</i>	55	25	70	58	70	55	75	60
13	<i>Fusarium moniliforme</i>	45	20	55	48	63	38	67	38
14	<i>Fusarium oxysporum</i>	35	15	22	08	50	40	37	20
15	<i>Macrophomina phaseolina</i>	00	00	00	00	05	10	00	07
16	<i>Penicillium spp.</i>	15	06	00	05	32	12	28	11
17	<i>Rhizopus stolonifer</i>	60	18	62	33	37	22	58	32

Table 1: Seed-borne fungal isolates of pulses Green gram, Black gram, Chickpea, Pigeon pea.

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