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ASSESSMENT OF SEED GERMINABILITY OF PULSES AGAINST INFESTATION BY SEED-BORNE FUNGI

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ABSTRACT
Seed mycoflora tend to affect adversely to germinability of seed. The vigour of the germinating seed is retarded by the seed-borne fungi. Assessment of the same is carried out in vitro by infestation of pulses seeds by their common seed-borne fungi. The common and dominant seed-borne fungi *Aspergillus flavus*, *A. fumigatus*, *A. niger* drastically reduced percent seed germination of the test pulses.

KEYWORDS: Seed germination, seed-borne fungi.

INTRODUCTION

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.) 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.) 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 phosphorous (312 mg) (Shakuntala Manay and M. Shadaksharaswamy,1987).

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).

Seed mycoflora of different categories of seeds such as bold, shrivelled, discoloured and cracked seeds have been studied. Sawhney and Aulakh (1980) studied fungi associated with normal and abnormal seeds of peas and their pathogenic potential. Ahmed *et al* (1981) studied mycoflora of cracked seeds of wheat and found that different fungi like *Penicillium* spp. *Aspergillus* spp. were responsible for the malformations of the test seeds. Randhawa and Aulakh (1984) studied mycoflora of discoloured and shrivelled seeds of pearl millet and found that seed mycoflora was responsible for the defect. Khairnar (1987) reported that, among different categories of mouldy seeds of bajra, the seeds with mixed type of discoloration were due to *Fusarium* spp. Tegge and Hiremath (1990) studied seed-borne fungi from shattering and non-shattering types of Green gram (*Vigna radiata* L.) and found that fungi associated were *Alternaria alternata*, *Cladosporium fulvum*, *Fusarium moniliforme*, *Aspergillus flavus*, *Aspergillus niger* and *Trichoderma roseum*. Further it is observed that, these fungi reduced seed germination and seedling growth. Danai (1994) reported that, the seeds of cracked and discoloured categories of jowar var. CSH-1 yielded maximum number of *Aspergilli* and also observed that, the bold seeds also showed fungi like *Aspergillus flavus* and *Aspergillus glaucus.* Umatale (1995) Studied groundnut and sunflower seeds and found that, discoloured seeds showed maximum incidence of fungi compared to wrinkled ones of Safflower and Sesamum. Waghmare (1996) Studied jowar var. CSH-1 seeds and found that, discoloured seeds were having highest counts of *Fusarium roseum* and *Fusarium semitectum*. Bodke (2000) studied different categories of seeds of cereals like wheat, bajra, jowar and maize and found that, discoloured seeds showed maximum number of fungi.

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. 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 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 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.

Effect of common and dominant seed-borne fungi of pulses on seed germination

In order to study the effect of common and dominant seed-borne fungi of pulses on the seed health of pulses, the test pulses like Green gram, Black gram, Chick pea and Pigeon pea were surface sterilized with 0.1% $HgCl_2$. These seeds were then washed repeatedly with sterilized distilled water to remove traces of $HgCl_2$. After washing, the seeds were separately treated with spore suspension of the common and dominant seed-borne fungi of pulses. Such artificially infested seeds were used to study percent seed germination, shoot, root length and percent seedling emergence separately. The seeds treated with sterile distilled water served as control.

Seed germination method

In order to evaluate the effect of seed-borne fungi on percent seed germination, the seeds of the test pulses were infested separately as mentioned above. These seeds were incubated in sterilized moist blotters at room temperature for ten days. After incubation period, percent seed germination, shoot and root length of each seeds of pulses was recorded.

Preparation of spore suspension

Spore suspension of common and dominant seed-borne fungi of pulses were prepared separately by adding 10 ml of sterile distilled water into the sporulating pure cultures of seed-borne fungi of pulses; maintained on PDA slants for seven days at room temperature. The slants were shaken and content was filtered through muslin cloth to separate mycelium and spore. The filtrate thus obtained was used as spore suspension.

RESULTS AND DISCUSSION

Results in the table show that seed-borne fungi retarded seed germination of the test seeds of pulses in variable quantity.

Table: Percent seed germination of Green gram (*Vigna radiata* L.), Black gram (*Vigna mungo* L.), Chickpea (*Cicer arietinum* L.) and Pigeon pea (*Cajanus cajan* L.).

Sr.No.	Common and dominant seed-borne fungi of pulses	Percent germination of test pulses			
		Green gram	Black gram	Chickpea	Pigeon pea
1	*Aspergillus flavus*	40	50	30	40
2	*Aspergillus fumigatus*	50	89	60	70
3	*Aspergillus niger*	30	30	40	60
4	*Drechslera tetramera*	60	60	20	80
5	*Fusarium moniliforme*	70	70	60	60
6	*Rizhopus stolonifer*	80	40	50	70
7	Control	90	90	100	90

The results reveal that, all common and dominant seed-borne fungi of Green gram retarded percent seed germination shoot and root length in the test seed of pulses. *Aspergillus niger* cause maximum reduction in seed germination (30%) followed by *Aspergillus flavus* (40%) and *Aspergillus fumigatus* (50%). On the contrary *Rhizopus stolonifer* and *Fusarium moniliforme* showed better seed germination (80% and 70% respectively).

The results indicate that, all the common and dominant seed-borne fungi of Black gram showed suppression in seed germination, shoot and root length of the pulse. The seed-borne fungus *Aspergillus niger* was most restrictive on seed germination showing only 30% seed germination (control 90%) followed by *Rhizopus stolonifer* (40%) and *Aspergillus flavus* (50%). *Aspergillus fumigatus* did not affect much adversely the seed germination compared to control. *Aspergillus flavus* caused much reduction in shoot length and completely inhibited growth of root.

Seed germination shoot and root length of the Chickpea adversely affected in more or less degree. *Drechslera tetramera* showed maximum retardation in seed germination (20%) followed by *Aspergillus flavus* (30%) and *Aspergillus niger* (40%). *Drechslera tetramera* completely inhibited shoot length, where as *Aspergillus niger* showed much reduced shoot length and much reduced root length.

Similarly, all common and dominant seed-borne fungi of Pigeon pea caused reduction in seed germination shoot and root length in variable degrees. Seed germination was much adversely affected by *Aspergillus flavus* (40%, control 90%), than *Fusarium moniliforme* and *Aspergillus niger* with both showing seed germination of 60%. Shoot, length was completely suppressed and root length was minimum in case of *Aspergillus niger*. However, there was increase in shoot length over control in case of *Drechslera tetramera* and increase in root length in case of *Aspergillus flavus* over control.

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