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Toxic Effects of Seed-Borne Fungi on Seed Health of Chickpea

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Abstract:

Green gram, Black gram, Pigeon pea and chickpea are common pulses in diet rich in carbohydrates, proteins and minerals. Numerous fungi affect pulses adversely causing reduction in seed content and seed health. During present study, effects of metabolites of common and dominant seed-borne fungi on seed health of Chickpea are evaluated. Total seventeen fungi recorded from all test pulses. Out of these seventeen seed-borne fungi, six, *Aspergillus flavus, A. fumigatus, A. niger, Drechslera tetramera* and *Rhizopus stolonifer*, found to be common and dominant on all four test pulses. These common and dominant seed-borne fungi produced mycotoxins that affected adversely to the seed germination, shoot and root length of test pulse Chickpea in variable quantity.

Keywords: seed-borne fungi, mycotoxins, test pulses.

1. Introduction:

Pulses are the second most important group of food plants belonging to family Leguminosae. They form an important and indispensable part of our daily diet. It is important source of dietary carbohydrates, proteins, essential amino acids and micronutrients such as calcium, phosphorus and iron. Therefore, pulses are important source of protein and essential amino acids for major vegetarians. The pulses like Green gram (*Vigna radiata* L.), Black gram (*Vigna mungo* L), and Chickpea (*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.

Various seed borne fungi affect pulses. Seventeen seed-borne fungi reported from all four test pulses i.e. Green gram, Black gram, Chickpea and Pigeon pea, of these six found to be common and dominant; they are *Aspergillus flavus, A. fumigatus, A. niger, Drechslera tetramera* and *Rhizopus stolonifer*. Metabolites i.e. mycotoxins of these fungi found to be adversely affecting to seed health of test pulse Chickpea.

Various researchers reported toxins of fungi and their effects on plants. Tripathi (1974) studied seed mycoflora of cereals and reported that, the culture filtrate of *Aspergillus flavus* was inhibitory to root and shoot growth. Deshpande and Gajewar (1976) studied seed mycoflora of cereals and found that the mycotoxins were causing adverse effects on seed germination. Kamal and Verma (1987) studied seeds of Arhar and reported that, seed germination was affected greatly due to *Aspergillus flavus, A. nidulans, A. niger, Trichoderma viridi* and *Alternaria alternata.* Sinha and Prasad (1981) reported adverse effects on seed germination of Mung due to *Alternaria alternata, Botryodiplodia theobrome, Curvularia lunata, Fusarium moniliforme* and *Macrophomina phaseolina*. Bodke (2000) studied toxins of seed-borne fungi in relation to different cereal seeds and found that, these seed-borne fungi adversely affected seed germination and seedling emergence. Kritzinger et al. (2003) observed *Fusarium proliferatum* produced mycotoxin in cowpea seeds and the toxin was reported to reduce seed germination. Gure et al. (2005) reported adverse affects of fungi on *Podocarpus falcatus* seeds; the toxins, caused reduction of seed germination, shoot length and root length. Owolade et.al. (2005) found about 100 secondary metabolites that are hazardous to maize seed produced by *Fusarium* spp. Howlett (2006) reported toxins of the seed-borne fungi found to be responsible to inhibit normal growth of seedlings in different crops. Culture filtrates of *Aspergillus* spp. caused reduction in seed germination and root-shoot elongation. (Jalandar and Gachande, 2012). Pathogenic fungi are known to produce mycotoxins such as oxalic acid crystals, kojic acid and malformins depending on growth conditions and the strains of organism (TSCA, 2012). Marked reduction in germination percentage and vigor index was reported in *Arachis hypogea* L. due to *Aspergillus flavus* (Naikoo Abaas et. al. 2013). Garuba et.al. (2014) reported reduction in percentage germination of maize seeds due to culture filtrates of *Aspergillus niger* and *Penicillium chrysogenum* isolated from maize seeds.

2. Materials and methods:

2.1 Preparation of spore suspension:

Spore suspension of six 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.

2.2 Crude toxin preparation:

Toxic metabolites of six common and dominant seed-borne fungi of pulses like, *Aspergillus flavus, A. fumigatus, A. niger, Drechslera tetramera, Fusarium moniliforme* and *Rhizopus stolonifer* prepared by growing fungi on liquid glucose nitrate (GN) medium and Chickpea seed flour medium (CPFM).

For this 25 ml of liquid glucose nitrate medium (GN) and Chickpea floor medium (CPFM) was poured separately in 100 ml borosil conical flasks and autoclaved. These flasks were then inoculated separately with 2 ml spore

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suspension of the test seed-borne fungi, which were maintained on PDA slants for seven days. These flasks were incubated at room temperature for ten days. After incubation, the culture filtrates were collected in pre-sterilized culture bottles from the flasks by filtering the cultures through Whatman filter paper No.1 and treated as crude toxin preparation.

2.3 Seed germination method:

Hundred seeds of test pulse Chickpea was soaked separately in crude toxin preparation i.e. culture filtrate (CF) of six common and dominant seed-borne fungi for 24 hrs. The soaked seeds were then placed on moist blotters in sterilized borosil glass Petri plates. The plates were incubated for 10 days at room temperature. After incubation, percent seed germination, root and shoot length were recorded. The seeds soaked in freshly prepared sterilized distilled water served as control.

2.4 Seedling emergence method:

Hundred seeds of test pulse Chickpea was soaked separately in crude toxin preparation, i.e. culture filtrate (CF) of six common and dominant seed-borne fungi for 24 hrs. The soaked seeds were sown at the depth of 2 cm equidistantly in earthen pots (25 cm diameter) containing sterilized soil at room temperature. Percent seedling emergence shoot length, root length were recorded after ten days. The seeds soaked in freshly prepared sterilized distilled water served as control.

Glucose nitrate (GN) medium:
Glucose: 10g
KNO_3: 2.5g
KH_2PO_4: 1g
$MgSO_4.7H_2O$: 0.5g
Distilled water : 1000 ml

Chickpea flour medium (CPFM):
Chickpea flour: 10g
KNO_3: 2.5g
KH_2PO_4: 1g
$MgSO_4.7H_2O$: 0.5g
Distilled water : 1000 ml

3. Results and discussion:

From the results presented in the Table it is evident that, toxins; i.e. culture filtrates obtained from all six common and dominant seed-borne fungi reduced seed germination, shoot and root length greatly. There was a much reduction in percent seed germination due to toxin of *Fusarium moniliforme* (GN) and *Aspergillus flavus* (CPFM) and least reduction was noticed from *Aspergillus fumigatus* and *Rhizopus stolonifer* (GN). Maximum reduction in root length was noticed from *Aspergillus niger* (GN) and *Drechslera tetramera* (CPFM), whereas least reduction was recorded from *Aspergillus fumigatus* (GN), Fusarium moniliforme, and *Rhizopus stolonifer* (CPFM). As regards to shoot length *Aspergillus flavus* (GN) and *Aspergillus niger* (CPFM) were most toxic, where as *Fusarium moniliforme* and *Rhizopus stolonifer* (CPFM) were found to be least toxic. Culture filtrates obtained from Chickpea floor medium (CPFM) was more toxic compared to culture filtrate obtained from Glucose nitrate (GN) medium.

Table: Effect of culture filtrate (CF) of common and dominant seed-borne fungi of pulses [grown on Glucose-nitrate medium (GN) and Chickpea floor medium (CPFM)] on seed health of Chickpea (*Cicer arietinum* L.) by blotter plate method (After ten days of incubation).

Sr. No.	Common and dominant seed-borne fungi	Seed germination					
		Seed germination (%)		Root length (cm)		Shoot length (cm)	
		GN	CPFM	GN	CPFM	GN	CPFM
1	*Aspergillus flavus*	30	20	4.8	3.5	02	02
2	*Aspergillus fumigatus*	50	50	5.2	03	4.8	02
3	*Aspergillus niger*	30	30	03	03	02	1.6
4	*Drechslera tetramera*	40	40	4.3	2.2	03	1.8
5	*Fusarium moniliforme*	20	40	4.3	4.6	05	2.3
6	*Rhizopus stolonifer*	50	40	05	4.6	04	2.3
7	Control	100	100	07	05	6.2	3.4

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