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FOUR HOST PULSES DEPENDENT PATHOGENIC VARIABILITY INCIDENCE OF FIVE ASPERGILLUS SPECIES FROM NANDED DISTRICT

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Abstract

Four test pulses Green gram (Vigna radiata L.), Black gram (Vigna mango L.), Chickpea (Cicer arietinum L.), Pigeon pea (Cajanus cajan L.) subjected to their common and dominant seed-borne fungi Aspergillus carbonarius, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger and Aspergillus nidulans. The virulence and pathogenicity of these fungi evaluated with the parameter of percent incidence of these five common and dominant seed-borne fungi on the test pulses. Out of five common and dominant pathogenic seed-borne fungi, Aspergillus flavus and A. niger found to be comparatively more pathogenic among all five common and dominant seed-borne fungi of test pulses.

Key words: *Aspergillus species, percent incidence, fungi*

Introduction: Pulses are important source of protein, carbohydrates, minerals and vitamins. Maharashtra produces Green gram, Black gram, Chickpea, Pigeon pea. These pulses are affected by different seed-borne fungi during germination stage. This infestation during incipient stage of crop growth adversely affects to the yield and quality of the pulse. In all seventeen fungi isolated from the four test pulses; six fungi found to be common and dominant. Five fungal species are from the genus *Aspergillus*. *Aspergillus* is responsible for the major damage to the quality and quantity of the pulses. *Aspergillus* mycotoxins are harmful for the consumers including humans. Five *Aspergillus* species i.e. *Aspergillus carbonarius*, *A. flavus*, *A. fumigatus*, *A. niger* and *A. nidulans* selected to study its infectivity on the test pulses to understand the comparative virulence of five species.

Pandy *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 Sithanantham (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. 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 and reported the virulence of these fungi on host plants.

Seed-borne fungi of pulses affect variably with variable percent incidence on their host pulses. Pathogenicity and occurrence of fungi depends upon toxigenicity of the individual fungus. *Aspergillus* is found to be one of the virulent genus of seed-borne fungi found on pulses i.e. Green gram (*Vigna radiata* L.), Black gram (*Vigna mango* L.), Chickpea (*Cicer arietinum* L.) and Pigeon pea (*Cajanus cajan* L.) under study. Abdulazia (2011) studied three local fungal isolates (*Aspergillus flavus, A. niger and A. ochraceous*) for their ability to secrete digesting enzymes (endoglucanase; β-galactosidase; β-mannanase; β-mannosidase and xylanase). The results revealed that the highest activity detected in the culture filtrate was for the β-mannanase enzyme followed by xylanase. Patil et.al. (2012) reported that highest percent incidence was due to *Aspergillus flavus*, followed by *A. niger*, *Penicillium notatum*, *Cladosporium* on Pigeon pea and Chickpea. Khairnar (2015) found forty one fungal species associated with seeds of Jowar, Maize, Bajra, Wheat and Paddy. *Aspergillus flavus* and *Curvularia pallescens* were found to be most pathogenic to the test crops. Rameela Chaudhari et.al. (2018)reported eight seed mycoflora (fungal species) namely, *Alternaria alternata, Fusarium oxysporum, Aspergillus niger, A. flavus, A. terreus, A. fumigatus, Macrophomina phaseolina and Phoma* sp. were isolated and used for study.

Materials and methods:

1) Collection of seed sample: The methods prescribed by Paul Neergaard (1977) have been adopted for the collection of seed samples. Seed samples of Green gram (*Vigna radiata* L.), Black gram (*Vigna mango* L.), Chickpea (*Cicer arietinum* L.) and Pigeon pea (*Cajanus cajan* L.) were collected from field, market places from Nanded. A composite seed sample for each of the pulse crop was made by mixing one kilogram of samples of the individual pulse together. These samples preserved in gunny bags at room temperature ($30^0C \pm 5^0C$) during the studies.
2) 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.

3) 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 seed were isolated and identified, brought into pure cultures and maintained on PDA (Potato Dextrose Agar) slants.
4) Detection and identification of seed mycoflora: The seed-borne fungi of different pulses detected by moist Blotter (B) and Agar (A) plate methods as recommended by ISTA (1966), De Tempe (1970), Neergaard (1977) and Agrawal (1981). The fungi identified on the basis of their morphological colony characters and reproducing bodies. Identifications of the fungi confirmed with the help of authentic manuals (Subramanian; 1971, Neergaard and Mathur; 1980, Jha; 1993 and Mukadam; 1997). Pure cultures of the identified fungi were made and maintained on PDA (Potato Dextrose Agar) slants.
5) Preparation of spore suspension: Spore suspension of five seed-borne fungi of pulses *Aspergillus carbonarius*, *A. flavus*, *A. fumigatus*, *A. niger* and *A. nidulans* 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.
6) Infestation of test pulses seeds with spore suspension: To study percent incidence of five *Aspergillus* species on the test pulses like Green gram, Black gram, Chick pea and Pigeon pea; the seeds were surface sterilized with 0.1% $HgCl_2$. And the seeds of the test pulses subsequently washed repeatedly with sterilized distilled water to remove traces of $HgCl_2$. After washing, the seeds were separately treated with spore suspension of the five seed-borne fungi of pulses. Such artificially infested seeds were incubated for ten days under aseptic conditions at 30ºC to 35ºC. The seeds treated with sterile distilled water served as control.

Results and discussion:

Results indicated in tables shows that comparative analysis of five seed-borne fungi *Aspergillus carbonarius, A. flavus, A. fumigatus, A. niger* and *A. nidulans* of test pulses cause variable pathogenic infestation to the test pulses. *Aspergillus flavus* found to be dominant in incidence among all five fungi with 60 % to 90 % incidence range, on the contrary *Aspergillus carbonarius* with least incidence ranging between 0 % to 29 % on the all test pulse.

Table 1: Percent (%) incidence pathogenic variability of five seed-borne *Aspergillus* species on their four host pulses.

Sr. No.	Five seed-borne species of *Aspergillus* from four test pulses	Green gram (%)		Black gram (%)		Chickpea (%)		Pigeon pea (%)	
		A	B	A	B	A	B	A	B
01	*Aspergillus carbonarius*	12	00	20	29	28	00	02	00
02	*Aspergillus flavus*	70	67	80	60	86	67	90	62
03	*Aspergillus fumigatus*	61	25	77	57	63	48	80	72
04	*Aspergillus niger*	60	55	90	67	82	70	78	52
05	*Aspergillus nidulans*	25	28	28	24	16	21	20	06

Where; A = (Agar plate), B = (Blotter paper)

Table 2: Average percent (%) pathogenic incidence of five seed-borne *Aspergillus* species on their four host test pulses.

Sr. No.	Five seed-borne species of *Aspergillus* from four test pulses	Green gram (A+B/2) (%)	Black gram (A+B/2) (%)	Chickpea (A+B/2) (%)	Pigeon pea (A+B/2) (%)	Total collective percent (%) incidence of each fungus on all test pulses
01	*Aspergillus carbonarius*	06	24.5	14	00	11.12
02	*Aspergillus flavus*	68.5	70	73.5	76	72.00
03	*Aspergillus fumigatus*	43	67	55.5	76	60.37
04	*Aspergillus niger*	57.5	78.5	76	65	69.25
05	*Aspergillus nidulans*	26.5	26	18.5	13	21.00

Where:

1) Average percent incidence = % incidence on Agar (A)+ % incidence on Blotter (B)/2 (i.e. number of media Agar and Blotter)

2) Total collective percent (%) incidence of each fungus on all test pulses = Summation of average percent (%) incidence of each fungus on all four test pulses/number of test pulses i.e. 4

Maximum average pathogenic percent incidence has been reported due to *Aspergillus flavus* (72%) followed by *Aspergillus niger* (69.25%). Whereas minimum pathogenic incidence shown by *Aspergillus carbonarius* (11.12%) on their host pulses.

It is found that *Aspergillus flavus* is most virulent among the five tested species on their four test pulses. The pathogenic variability of the different species of *Aspergillus* could be attributed to the host pathogen interaction. That is pathogenesis is the process of attack on the part of pathogen and defense on the part of host leading to overall actions and reactions between two antagonistic organisms. The pathogenic ability of *Aspergillus flavus* and *A. niger* attributed to their cellulolytic, lignolytic and toxigenic activities. The endotoxins Aflatoxin B1 and B2 secreted by *Aspergillus flavus* and Fumonisin B2 and B4 by *A. niger* are more virulent compared to other species of *Aspergillus* genus.

Mei-Tsu Shieh et.al. (1997) reported that, polygalacturonase P2c contributes to the invasion and spread of *A. flavus* during infection of cotton bolls. Hedayati et.al. (2007) showed toxicity of *Aspergillus flavus* by invasive infections in mice to be 100-fold more virulent than *A. fumigatus* in terms of inoculum required. In addition, *A. flavus* produces aflatoxins, the most toxic and potent hepatocarcinogenic natural compounds ever characterized. Rocio et.al. (2007) experimentally proved; the plant pathogenic fungus *Aspergillus flavus* produces several types of mycotoxins. The most well known are the carcinogenic compounds called aflatoxins. In addition, *A. flavus* produces cyclopiazonic acid and aflatrem mycotoxins, contributing to the toxicity of *A. flavus* infected crops. Roopam Parashar et.al. (2019) found thirteen genera and twenty three species on Chickpea, Green gram, Pigeon pea and Lentil, among all Chickpea showed maximum percent of seed-borne fungi. Eleven seed-borne fungi namely; *Aspergillus flavus*, *A. niger*, *Trichoderma* sp., *Fusarium* sp., *Alternaria* sp., *Mucor* sp. etc. were reported by Shekhar Kumar et.al. (2017) and found that seed-borne fungi has inverse proportion to seed germination percentage of five pulses. Vasava et.al. (2018) found seven major seed-borne fungi on Cowpea cultivars among these *Fusarium oxysporum* was more adversely affected to seed germination, seedling emergence and seedling vigour. Halla H. Abd-Allah et.al. (2018) studied four crops of Fabaceae family Beans, Lupine, Pea and Soybean for their seed-borne fungi. They found eleven fungal species like *Alternaria alternata, Aspergillus flavus, A. niger, A. parasiticus, Aspergillus terreus, Epicocum* sp., *Fusarium* spp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp. and *Trichoderma* sp. were isolated and identified from tested beans (*Phaseolus vulgaris* L.),

Lupine (*Lupinus termis* L.), pea (*Pisum sativum* L.) and soybean (*Glycine max* L.) seed samples. Toxic effect of filtrates of these fungi has different degrees of deterioration including decreases viability of seeds (germinability), protein, lipids, carbohydrates and energy value than control.

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