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Seed mycoflora of some pulses collected from Bundelkhand region

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Abstract
The present paper deals with a study to identify the fungi associated with seed of four major pulses of Bundelkhand namely Chickpea, Mung bean, Pigeon pea, and Lentil. Isolation of seed mycoflora was done by both blotter and Agar technique prescribed by ISTA 1996. Both the sterilized and unsterilized seed were taken. Further the effect of seed mycoflora on the germination percent of the seeds was estimated. In our study 13 genera and 23 species were isolated. There was variation in total CFU and number of seeds associated with these pulses. Chickpea possessed maximum number of seed fungi. As well as showed its superiority in germination percentage among the other legumes.

Keywords: ISTA method, agar plate technique, blotter paper technique, pulses, seed mycoflora

Introduction
Seeds are the starting and end product of every crop and are the passive carrier of pathogens. A viable seed is a source of the new plant. Microorganism plays a important role in effecting the quality of seed in which fungi are the largest group. Fungi associated with seed cause reduction of plant growth and productivity of crop. The legume or pulses belongs to family Fabaceae. The high protein content makes them desirable crop in Agriculture. They stand second only to cereals. Pulses are of high economic value because of the rich source of protein and contain carbohydrate, dietary fiber and minerals (Ofuya and Akhidue, 2005) [24]. Pulse crops also improve soil fertility by fixing atmospheric nitrogen.
Pulses, supplemented with cereals, provide a perfect mix of vegetarian protein of high biological value. India is the largest producer, importer and consumer of pulses, accounting for 25% of global production. The persistent and growing demand–supply gap is putting pressure on prices and this good source of vegetarian protein is turning inaccessible to the poor. Pulses are an integral component of sustainable crop-production as they have ability of biological nitrogen fixation, low water requirement and a basic ingredient in the diet of vast majority of poor and vegetarian population in India **(**Ahlawat *et al*, 2016) [1].
The major pulse crop grown in Bundelkhand are Chickpea (*Cicer arietinum* L.), Mung bean (*Vigna radiate* L.), Pigeon pea (*Cajanus cajan* L.) and Lentil (*Lens esculenta* Medik.) Several factors are responsible for their low production. There is need in the field of sustainable agriculture practices to improve the yield of pulse crop. Among these factors disease plays a major role (Nine, 1986) [22] and (Pal, 1996) [25]. Seed born diseases can causes economic crop losses; reduction in plant growth and productivity of the crop. (Kubiak and Korbas, 1999) [17]; (Dawson and Bateman, 2001) [8]; (Islam *et al.*, 2009) [14], and (Weber *et al.*, 2001) [31], Presence or absence of seed fungi on seed surface is one of the major aspects that reduce the quality of seed. The present investigation was aimed to estimate the fungi associated with the seeds of selected pulses of Jhansi and Banda district of Bundelkhand.

Material and Methods
Plant material
Seed of four pulses Chickpea Mung bean, Pigeon pea, and Lentil, were collected from local market of different villages and certified Seed center of Jhansi and Banda, district of Bundelkhand (UP).

Seed mycoflora analysis
Fungi associated with four pulses (Chickpea, Mung bean, Pigeon pea, and Lentil) was studied by Blotter Method and Agar Method. Seeds were analyzed for seed mycoflora following standard method of International Seed Testing Association (ISTA, 1996). Experiment was carried out with both sterilized (S) and unsterilized (US) seeds. Seed simply washed with distilled water were used as unsterilized (US) seeds.

Seeds which were surface sterilized with 0.1% Sodium hypochlorite for 10 minutes and rinsed with distil water 2 to 3 times used as sterilized seeds (S). The sterilized and unsterilized seeds were separately placed onto petri plates having three layers of moistened blotter paper. Ten seeds were taken in each petri plate. Arrangement of the seeds was as per ISTA rule. Overall 100 seeds were taken for each case. PDA (Potato dextrose media) was prepared as per to (Aneja, 1996). The sterilized and unsterilized seeds were separately placed onto petri plate having P.D.A. The plates were incubated in incubator at 25± 2˚C for 7 days. The observations were regularly recorded at 24 hr. interval for the presence of seed fungi, total cfu and the germination. The unsterilized seed were taken as control, experiment was repeated thrice. The initial observation of the fungal growth were taken after 72 hrs and concluded on seventh day. The different colony of fungi were estimated and further isolated for identification. The identification of the fungi was done by (Alexopolus *et al.*, 2017) [3].

Seed germination

Germination percent of the was calculated according to the below given formula.

$$\text{Germination \%} = \frac{Total\ no\ of\ germinated\ seed}{Total\ no\ of\ seeds\ (Plate)} \times 100$$

Purification and identification

The Identification of the isolated fungi associated with the seeds was primarily done on the basis of colony characteristics that are the color, shapes, and size of the culture and reverse plate of colony. The isolated fungi were identified with the help of the keys, monograph and literature provided by (Raper and Fennell 1965, Booth 1971, Ellis 1971, Nelson *et al*., 1983, Barnett and Hunter 1972, Domsch and Gams 1980 and Alexopolus *et.al.*, 2017) [26, 7, 17, 21, 6, 11, 3] and authentic manuals (Subramanianm, 1971, Neergaard and Mathur, 1980, Jha, 1993 and Mukadam, 1997) [29, 20, 16, 19].

Results and Discussion

Seed play a vital role in the production of healthy crops. Healthy seeds are the foundation of healthy crop and are an assurance of good yield (Diaz *et al.*, 1998) [10]. In our study it was visualized that seed born fungi have a negative impact on the germination of taken pulses. In this study total 23 species were found associated with the seed of four important pulses. These sp. belonged to 13 genera (Table 1). The maximum number of fungal species were isolated from the unsterilized seed of Chickpea followed by Mung bean and Pigeon pea. Least fungal species were isolated from Lentil. Among fungal flora majority of species were of genera *Aspergillus*. followed by *Fusarium, Trichoderma., Mucor, Curvularia, Alternaria, Botrytis, Rhizopus, Cladosporium, Drechslera, Macrophomina, Pythium., Chaetomium* and unidentified species. The overall study revealed that *Pythium sp.* was obtained only from lentil. The sterilized seed harbored less fungal flora as compared to unsterilized seed. On other hand the fungal population was higher in blotter technique in comparison to agar technique.

In our this study *Aspergillus carbonarius*, *A. niger* van Tieghem, *A. fumigatus* Fresenius, *A. flavus* Link ex Gray, *A. tenuis*, *Alternaria alternata* Keissler, *Botrytis cineria*, *Chaetomium globosum* kuhze ex steud, *Cladosporium sp*, *Curvularia lunata* Boedijn, *Drechslera sp*., *Fusarium soloni* (Mart.) Sacc., *Fusarium oxysporum cicris, F.udam, F. oxysporum* Schle. ex Fries, *Mucor sp.* Mich. Ex St.-Am., *Macrophomina phaseolina* (Tassi), *Pythium sp.*, *Rhizopus stolonifer* (Ehrenb. Ex Link) Lind, *Trichoderma harzianum, T. viride Persoon* ex Fries, *T. hamatum* (Bonord.) Bainier etc. were obtained from undertaken pulses. (Ghangaoker *et al.* 2013) [13] obtained similar observations by blotter technique from the sterilized and unsterilized seeds of *Pisum sativum*, Lens, *Cajanus cajan, Cicer arietinum*, Lens *esculenta, Phaseolus vulgaris* and *Vigna unguiculata* in Pune.

We observed that the most common genera of fungi was *Aspergillus niger*, *Alternaria*, *Curvularia*, *Fusarium* and *Trichoderma* as they were present in all selected legumes. In chickpea the incidence of *Aspergillus niger, Fusarium oxysporum, Fusarium oxysporum cicri, R. stolonifer* and *Trichoderma viridi* was high. Similar observation were recorded by Ahmad *et al., (*1993) [2] while working on seed mycoflora of chickpea. Sarita *et al.* (2014) [28] isolated seed mycoflora of mung bean and obtained probably same genera and species of fungi as in our study with the exception of *Helminthosporium* and *Penicillium* was present in their study and *Trichoderma* were present in our study.

The unsterilized seeds of pigeon pea possessed nine genera and 13 sp viz,. *Aspergillus. niger, A. flavus, A. fumigatus, Alternaria alternata, Botrytis cineria, Chaetomium globosum, Drechslera sp, Mucor sp, Fusarium oxysporum, F. udam, Trichoderma harzianum, and T. viride.* (Arya and Mathew 1991) [5] isolated the same flora from pigeon pea. The sterilized seeds of Lentil harbored viz., *Aspergillus niger, A. flavus, Fusarium soloni, Pythium sp, Rhizopus stolonifer and T. viride* by blotter paper. (Muhammad *et al*, 2007) [18] in their study isolated *Penicillium citrinum, Aspergillus flavus, A. terreus* and *Nigrospora sp.* besides our isolated fungi from unsterilized seeds of Lentil. The factors responsible for these changes may be due to viability and maturity of seed and the environmental conditions. (Table 2 and Graph 2) revels the result of CFU (Colony forming unit) and germination percentage the maximum CFU (32) were found in the unsterilized seed of chickpea and minimum CFU(5) were obtained from sterilized seed of Lentil. The presence of fungi were visualized higher in unsterilized seed with Blotter technique in comparison sterilized seed in Agar technique.

During the present study it can be concluded that infected seed leads to poor germination which ultimately result in low germination percentage or unhealthy crop. The sequence of viability was *Cicer arietinum* L.> *Vigna radiata (*L.) Wilczek > *Cajanus cajan* L. Millsp. >*Lens esculenta* Medikus. The large number of fungi is if associated with seed it causes deterioration of seeds and affect viability (DGISP 1985). (Umechuruba and Nwachukwu, 1994 and Nwachukwu and Umechuruba 1997) [30, 23] in their study have reported that seed born fungi is responsible for the significant reduction seed germination, seedling emergence, low seed and low yield and finally are responsible for the reduction in nutritional qualities of the seeds. Further studies on proper upkeep of the pulses in storage and technologies for treating the seeds in required to meet out the pulses demand of our nation.

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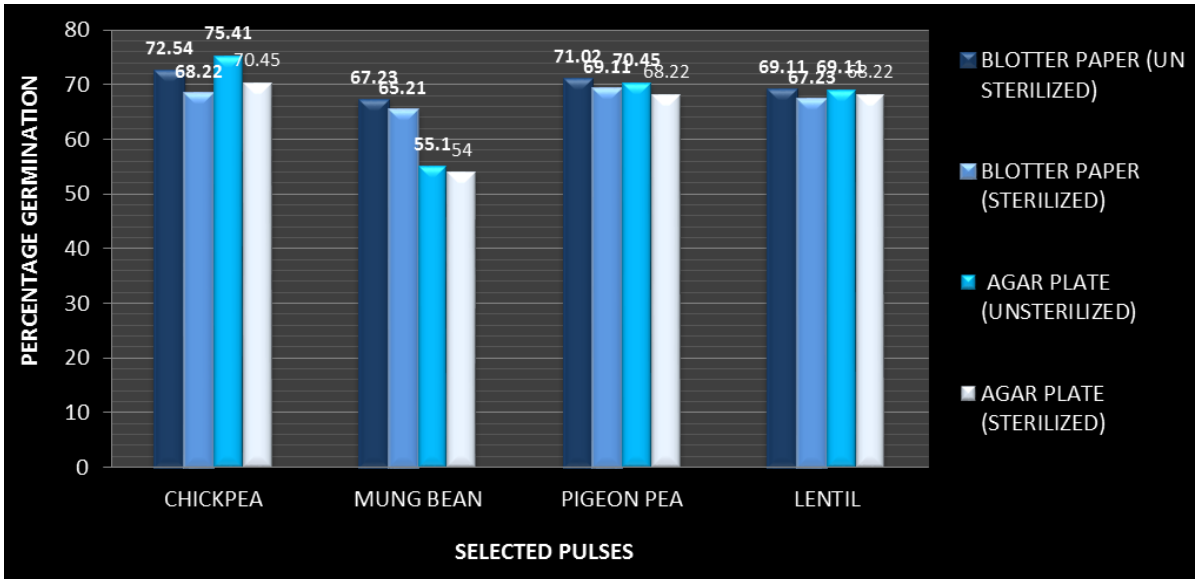
Table 1: Seed mycoflora isolated through ISTA technique from selected four legumes seeds

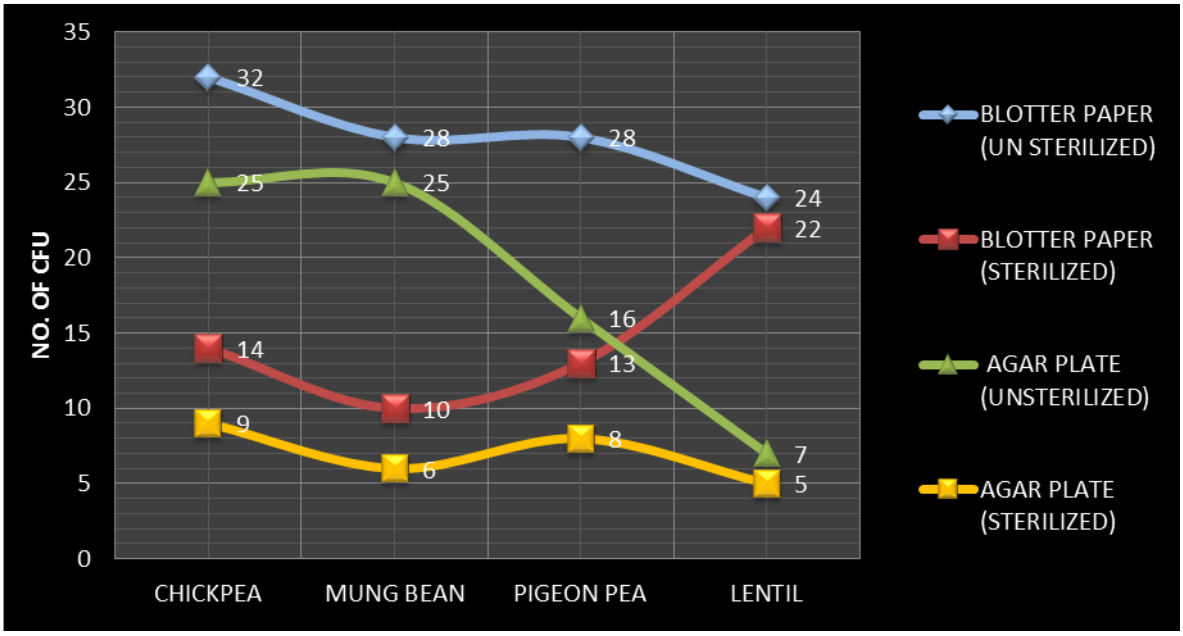
S. No	Seed Mycoflora	Chickpea				Mung Bean				Pigeon Pea				Lentil			
		BP		A P		BP		A P		BP		A P		BP		A P	
		US	S	US	S	US	S	US	S	US	S	US	S	US	S	US	S
1.	*Aspergillus carbonarius*	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	*A. niger van Tieghem*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.	*A. fumigatus Fresenius*	+	-	+	-	-	-	+	-	+	+	+	-	-	-	+	-
4.	*A. flavus* Link ex Gray,	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5.	*A. tenuis*	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
6.	*Alternaria alternata* Keissler	+	+	+	-	+	-	-	-	+	-	+	-	+	-	-	-
7.	*Botrytis cineria.*	+	-	+	-	+	-	+	-	+	-	+	-	-	-	+	-
8.	*Chaetomium globosum* kuhze ex steud	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
9.	*Cladosporium sp.*	+	-	+	-	+	-	-	-	-	-	-	-	+	-	+	-
10.	*Curvularia lunata* Boedijn	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-
11.	*Drechslera sp.*	-	-	-	-	+	-	+	-	+	+	+	-	-	-	-	-
12.	*Fusarium soloni* (Mart.) Sacc.	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	+
13.	*Fusarium oxysporum cicris*	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
14.	*F. oxysporum* Schle. ex Fries	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+
15.	*Fusarium udum*	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
16.	*Mucor sp.* Mich. Ex St.-Am.,	+	+	+	+	+	+	+	-	+	-	+	-	+	-	+	+
17.	*Macrophomina phaseolina* (Tassi)	+	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
18.	*Pythium sp*	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
19.	*R. stolonifer* (Ehrenb. Ex Link) Lind,	+	+	-	-	+	+	-	-	+	+	-	-	-	+	-	-
20.	*Trichoderma harzianum*	+	-	-	-	+	+	+	-	+	-	-	-	+	-	+	-
21.	*T. viride* Persoon ex Fries	+	+	+	-	+	-	-	-	+	-	+	-	+	+	-	-
22.	*T. hamatum* (Bonord.) Bainier	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-
23.	*Unidentified*	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-

Table 2: Percentage of seed germination results of selected legumes seeds.

S. No	Pulses	Blotter				Agar			
		Unsterilized		Sterilized		Unsterilized		Sterilized	
		Germination %	CFU	Germination %	CFU	Germination %	CFU	Germination %	CFU
1	Chickpea	72.54	32	68.22	14	75.41	25	70.45	9
2	Mung Bean	67.23	28	65.21	10	55.1	25	54.00	6
3	Pigeon Pea	71.02	28	69.11	13	70.45	16	68.22	8
4	Lentil	69.11	24	67.23	22	69.11	7	68.22	5



Graph 1: Percentage of seed germination results of selected legumes seeds.



Graph 2: No of CFU of selected pulse.

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