RESEARCH ARTICLE

Mycoflora associated with seeds of chickpea

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ABSTRACT

In the present investigation, fungi associated with chickpea (*Cicer arietinum* L.) were detected by blotter paper and agar plate method. Thirteen different fungi like *Actinomucor repens, Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, A. ochraceus, Cladosporium sp., Fusarium oxysporum, Fusarium sp., Mucor varians, Penicillium notatum, Phoma herbarum, Rhizopus stolonifer* were isolated in variable frequencies. Frequency of the individual species ranges between 1.11 – 8.19%. Of which, *Fusarium oxysporum* (8.19%), *Rhizopus stolonifer* (7.63%), *Phoma herbarum* (5.69%) and *Aspergillus flavus* (5.44%) were found to be predominant. Blotter paper method was found to be more effective than agar plate method. The percent germination of the Chickpea seeds was evaluated by the standard rolled paper towel method. Higher incidence of fungi on the seeds of chickpea adversely affected its germination.

Key words: Seed-borne mycoflora, % frequency, % germination, and Chickpea.

INTRODUCTION

Plants play an important role in the lives of human being. They are an essential resource for human well-being and also a source of energy. Pulse plants which are rich source of energy and are largely cultivated and consumed in India. *Cicer arietinum* L. which is also known as chickpea is the world's third most important grain legume globally grown in over 40 countries (Anwer *et. al*., 2009) and is one of the most important pulse crop grown in India. About 65 % of the global area with 68% of global production of chickpea is contributed by India (Reddy and Mishra, 2010). It is the most nutritive pulse crop extensively used as protein adjunct to starchy diet. Out of which digestibility of protein varies from 76-78 % and its carbohydrates from 57-60 %. The seeds contains essential amino acids, mineral, fibers, unsaturated fatty acids and β- carotene (Jukanti *et. al.,* 2012).

Seeds are the basic input for crop production. Pathogen free healthy seeds are required for healthy and high yield crop production. Although chickpea is known for its excellent source of nutritional and agronomical value. But there are lots of factors involved in the yield loss in which fungi play an important role. Many plant pathogens are seed-borne, which can cause enormous crop losses; reduction in plant growth and productivity of crops (Islam *et al.,* 2009). The seed borne pathogens associated with seeds externally or internally may cause various infection viz., seed rot, seed necrosis, reduction

or elimination of germination capacity, as well as seedling damage resulting in development of disease at later stages of plant growth by systemic infection (Khanzada *et al.,* 2002).

Chickpea is known to attack by about 67 fungi, 3 bacteria, 22 viruses and mycoplasma and 80 nematodes (Nene *et. al*., 1996). Many fungal species viz.., *Alternaria porri*, *A. alternata*, *Aspergillus amstelodami*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. sydowi*, *A. wentii*, *Botrytis cinerea*, *Cladosporium macrocarpum*, *Curvularia lunata*, *Fusarium equiseti*, *F. moniliforme*, *F. oxysporum*, *F. semitectum*, *Macrophomina phaseolina*, *Myrothecium roridum*, *Penicillium notatum*, *Rhizoctonia sp*., and *Rhizopus arrhizus* been reported from chickpea (Ahmad *et*. *al*., 1993). Many workers have detected different mold fungi and their toxin production ability in stored grains which deteriorate the stored products (Afzal *et*. *al*., 1979). Also, the seasonal climatic variation of Vidarbha and improper storage condition contribute to make the storage environment extremely supportive for fungal attack to the nutrient rich seeds (Bhajbhuje, 2013). Therefore the present study was conducted to isolate seed fungi and its effect on seed germination.

MATERIALS AND METHODS

The seed samples of chickpea (*Cicer arietinum* L.) collected from five different talukas of Amravati district during 2010-2011, were brought to laboratory in sterile cotton bags and kept at room temp. The untreated seeds were used for isolation of external mycoflora while surface sterilized seeds by aqueous 0.1% mercuric chloride solution were used for detection of internal seed mycoflora. The isolation of seed mycoflora was made by standard blotter paper and agar plate method technique of ISTA (2012). After incubation for seven days at 25±1^{0}C, seeds were observed under stereo-binocular microscope for prevalence of fungal growth on seed surface. A count of germinating seeds as well as fungal colonies on seeds was taken and expressed in percent frequency (Bhajbhuje, 2013). The fungal isolates were sub-cultured in slants, identified using keys and manuals (Neergaard, 1977).

RESULTS AND DISCUSSION

Mycological analysis of the seed samples of chickpea revealed the prevalence of total 9 genera belonging to 13 species with varying frequency.

Table 1 : Frequency of isolated fungi on chickpea.

S. N.	Fungi isolated	January-12		February-12		March-12		Mean±SD	±SE
		E	I	E	I	E	I		
1	*Actinomucor repens*	-	-	-	-	11.66	-	1.94±4.76	±1.95
2	*Alternaria alternata*	-	3.33	0.83	5.83	1.66	5	2.77±2.77	±0.95
3	*Aspergillus flavus*	7.5	11.66	10.83	3.33	8.33	-	5.44±4.49	±1.84
4	*Aspergillus fumigatus*	1.66	2.5	3.33	1.66	3.33	3.33	2.63±0.82	±0.33
5	*Aspergillus niger*	3.33	-	9.16	1.66	1.66	6.66	3.74±3.48	±1.42
6	*Aspergillus ochraceus*	4.16	-	3.33	-	5.83	-	2.22±2.56	±1.04
7	*Cladosporium* sp.	-	-	4.16	2.5	5.83	-	2.08±2.51	±1.02
8	*Fusarium oxysporum*	10	14.16	-	-	10.83	14.16	8.19±6.56	±2.68
9	*Fusarium sp.*	-	-	6.66	-	-	-	1.11±2.71	±1.11
10	*Mucor varians*	2.5	-	2.5	2.5	-	13.3	3.46±4.97	±2.03
11	*Penicillium notatum*	1.66	4.16	-	11.66	-	1.66	3.19±4.42	±1.81
12	*Phoma herbarum*	5.83	10	9.16	-	9.16	-	5.69±4.63	±1.89
13	*Rhizopus stolonifer*	14.16	2.5	10	15	4.16	-	7.63±6.31	±2.58
Total		50.8	48.31	59.13	44.14	62.45	44.11		
		99.11		103.27		106.56			

S.D. = ±Standard deviation; S.E. = ± Standard error

Table 2 : Germination % of Chickpea seeds.

Sr. No.	Month	Germinated seeds (%)	Non-germinated Seeds (%)
1.	January-2012	91.33%	8.67%
2.	February-2012	89.33%	10.67%
3.	March – 2012	86%	14%

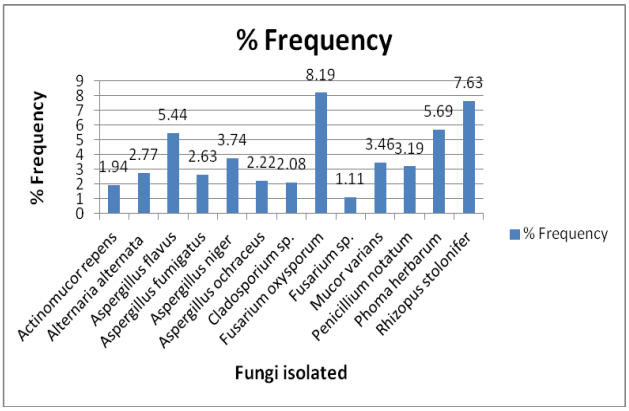

Fig.1: Frequency of individual fungal species on chickpea seeds

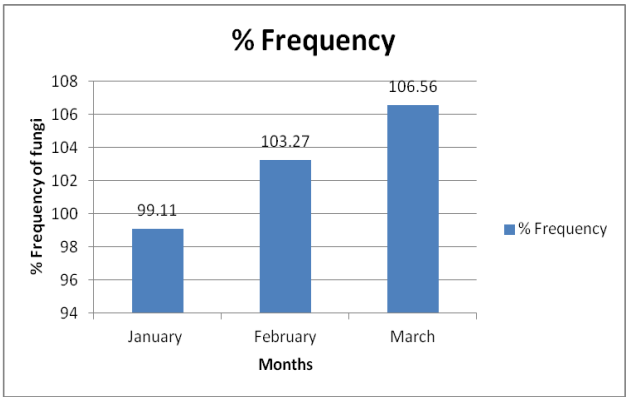

Fig 2:Frequency of fungi with respect to month.

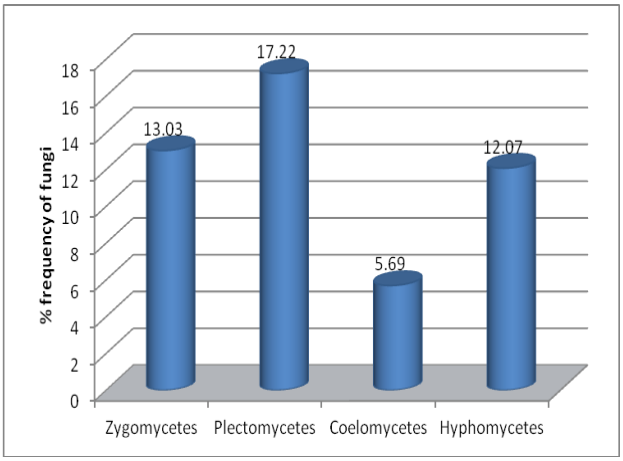

Fig. 3: Distribution of fungal flora with respect to classes

In the present investigation, *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium sp., Fusarium oxysporum, Penicillium notatum, Phoma herbarum, Rhizopus stolonifer* were commonly isolated by both the methods on chickpea seeds whereas, *Actinomucor repens, Aspergillus ochraceus* and *Fusarium sp.* were isolated only by standard blotter paper method. Singh (2014) also isolated seven fungal species such as *Alternaria alternata*, *Aspergillus flavus* , *A. niger*, *A. fumigatus*, *Curvularia lunata*, *Fusarium monoliforme* and *Rhizoctonia solani*. Ghangoaker and Kshirsagar (2013) also reported many fungal species *viz. Alternaria alternata*, *Aspergillus terrus*, *A. flavus*, *A. fumigatus*, *A. niger*, *Botrytis sp*., *Cladosporium*, *Curvularia lunata*, *Fusarium solani*, *F. moniliforme*, *F. oxysporum*, *Macrophomina phaseolina*, *Penicillium notatum*, *Rhizoctonia sp*. and *Rhizopus nigricans* from *Cicer arietinum* L.

The predominant fungi detected in the order of prevalence were found to be *Fusarium oxysporum* (8.19%), *Rhizopus stolonifer* (7.63%), *Phoma herbarum* (5.69%) and *Aspergillus flavus* (5.44%). Similar observation was recorded by Ghangoaker and Kshirsagar (2013). Amongst the 13 isolated fungi from chickpea, Plectomycetes contributed 2 genus and 5 species followed by Hyphomycetes with 3 genus and 4 species; Zygomycetes with 3 genus and 3 species. Coelomycetes had single genus and species. Hedawoo *et al*., (2014); Bhajbhuje (2013) reported higher count of fungal isolates in Ascomycota from spices seeds. Altogether a population of 13 fungal species was isolated by blotter paper method, exhibited greater fungal count over agar plate (Table 1). It is revealed that blotter paper method was found to be more effective over agar plate and also surface sterilization reduce fungal incidence. It is in agreement with earlier report of Bhajbhuje (2013) who recorded greatest count of fungal isolates by blotter paper method against agar plate. In each month, frequency of external mycoflora was greater than internal mycoflora.

The results presented in table 2, revealed 91.33% seed germination of chickpea in January, 89.33% in February and 86% in March. It was observed that, increase in the fungal frequency, decreases the per cent germination. Javaid (2005) also pointed out that heavy fungal infestation on the seeds of chickpea that adversely affected its germination.

CONCLUSION

Chickpea because of its high protein contain secure an important position amongst the pulse crops. But these seeds in the storage condition become more susceptible to fungal infection resulting in the lowering in seed germination and deterioration in storage. A damage seed will produce an abnormal seedling. Thus farmers are advice to use pathogen free healthy seeds to overcome the losses in productivity. But without seed health test it is not possible to detect healthy seeds for better productivity.

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