INT. J. BIOL. BIOTECH., 16 (1): 175-179, 2019.

MYCOFLORA ASSOCIATED WITH SEEDS OF DIFFERENT VARIETIES OF CHICKPEA AND ITS EFFECT ON GERMINATION AND RADICLE GROWTH

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ABSTRACT

Fungi associated with four chickpea (*Cicer arietinum* L.) varieties namely Bhakar, Thal, Noor and Punjab were identified using blotter paper method. Nine fungal species namely *Alternaria alternata* (Fr.) Keissl., *Aspergillus flavus* Link, *Aspergillus niger* van Tieghem, *Aspergillus oryzae* (Ahlburg) E. Cohn, *Fusarium oxysporum* Schlecht. Emend. Snyder & Hansen, *Macrophomina phaseolina* (Tassi) Goid., *Mucor* sp., *Penicillium italicum* Wehmer and *Sclerotium rolfsii* Sacc. were found associated with the seeds of selected chickpea varieties. The highest fungal incidence (100%) was recorded in Noor where all the seeds were found fungal contaminated followed by Thal (48%), Bakhar (44%) and Punjab (42%). Seed germination in surface-sterilized and non-sterilized seeds ranged from 96–100% and 88–96%, respectively. The difference in germination between surface-sterilized and non-sterilized was insignificant in all the varieties except Thal. However, radicle length was significantly lower in non-sterilized seeds as compared to sterilized ones. The highest value of correlation coefficient (r = -0.95) was recorded between *P. italicum* and germination followed by r = -0.72 between *S. rolfsii* and radicle length.

Key words: Chickpea varieties, correlation, germination, radicle length, seed mycoflora.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the earliest cultivated crops that are consumed all over the world (Tripathi *et al*., 2015). It is one of the most important pulses that are considered to be better than others due to their nutritive values. It is commercially grown in tropical, sub-tropical, semi-arid and Mediterranean regions of the world (Kashiwagi *et al*., 2015; Kandhare, 2015). Pakistan is the 2nd largest chickpea growing and third biggest chickpea producing country in the world with an average yield of 561 kg. ha^{-1}, where the crop is grown as the principal winter pulse crop, fodder and green manure (Imran *et al*., 2015). Among food legumes, it is a traditional source of proteins and carbohydrates containing significant amounts of all the essential amino acids (Barakat *et al*., 2015). Chickpea seed has 58.9% carbohydrate, 3% fiber, 5.2% oil, 3% ash, 0.2% calcium, and 0.3% phosphorus (Motamedi *et al*., 2015). It is also a main source of important vitamins such as riboflavin, niacin, thiamin and folate (Hameed *et al*., 2015).

Presence or absence of mycoflora on seed surface is one of the important aspects that determine the quality of seed. More than 50 pathogenic and stored grains fungi have been reported to affect chickpea particularly, *Ascochyta rabiei* (Pass.) Lab., *Botrytis cinerea* Pers., *Sclerotium rolfsii*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *A. oryzae*, *Penicillium italicum* and *Mucor* sp. present on the seed surface (Kaur *et al*., 2015; Muhammad *et al*., 2015; Leo *et al*., 2015; Kandhare, 2015; Zaidi and Pathak, 2015). In the present study, four varieties of chickpea were studied to check seed-borne mycoflora and its relations with seed germination and radicle growth.

MATERIALS AND METHODS

Seed samples of four economically important chickpea varieties namely Bhakar, Thal, Noor and Punjab were used for the isolation of seed microflora. The isolation of seed-borne fungi was carried out by using blotter paper method as it is a useful incubation method for the detection of deep-seated pathogens. Seeds were surface sterilized by 3% sodium hypochlorite solution for 5 minutes then washed 3 times with sterilized distilled water. After washing 10 seeds were placed at equal distance in each Petri plate having filter paper moist with 4 mL of autoclaved distilled water. Non-surface sterilized seeds were placed in separate Petri plates using the same method. Both surface-sterilized and non-sterilized seed treatments were prepared five times. Petri plates were kept at room temperature for 72 h. After incubation, fungi developed on seeds were transferred on malt extract agar plates. Pure fungal cultures were examined under different magnification of a stereomicroscope and identified. Percentage incidence of each

fungal species was also calculated. The fungi were identified on the bases of their morphological and cultural characteristics as available in literature (Domsch *et al.,* 1980; Klich, 2002; Simmons, 2007; Bennett, 2010).

Seeds were allowed to germinate for 14 days. Data regarding germination and radicle length were recorded and analyzed statistically by apply ANOVA followed by LSD test using computer software Statistix 8.1. Correlation of fungal flora (%) of chickpea with germination and radicle length in non-sterilized seeds was evaluated using MS Excel program.

RESULTS AND DISCUSSION

Fungi appeared both on surface sterilized as well as non-treated seeds. However, fungal incidence was significantly higher in non-treated as compared to surface sterilized or treated seeds in all the four chickpea varieties. Fungal incidence ranged from 4–18% in surface sterilized while it ranged from 42–100% in non-treated seeds. The highest fungal incidence in treated and non-treated seeds was recorded in chickpea var. Noor that was 18% and 100%, respectively, and was significantly higher as compared to fungal incidence in other three tested varieties (Fig. 1A). Variation in incidence of seed-borne mycoflora of chickpea has also been reported by Warude *et al.* (2016).

A total of nine fungal species including both pathogenic and storage fungi were found associated with seeds of different chickpea varieties. These included *Alternaria alternata, Aspergillus flavus, A. niger, A. oryzae, Fusarium oxysporum, Macrophomina phaseolina, Mucor* sp., *Penicillium italicum* and *Sclerotium rolfsii.* Among these, *Mucor* sp., *P. italicum, F. oxysporum* and *A. alternata* were more abundant than rest of the fungal species (Table 1). Among the identified species, *M. phaseolina, S. rolfsii, F. oxysporum* and *A. alternata* are pathogenic fungi and cause root rot, collar rot, wilt and blight diseases, respectively, in chickpea (Mandhare *et al.,* 2008; Ali *et al.,* 2017; Khan *et al.,*2017; Saabale *et al.,* 2017). *Aspergillus* spp., *Mucor* sp. and *P. italicum* are storage fungi. Previous literature shows that fungi associated with seeds of chickpea may vary depending on the chickpea variety, area of seed collection and storage conditions. Recently, Kushwaha (2017) isolated ten fungi from non-treated chickpea seed namely *Aspergillus candidus, A. niger A. flavus, Curvularia lunata, B. cinerea, Dresclera rostrata, Rhizopus arrhizus, Chaetomium globosum, F. oxysporum* and *Mucor varians*. Some of these fungal species were also found in the present study. Warude *et al.* (2016) identified 8 fungal species belonging to six genera namely *A. alternata, A. niger, A. flavus, F. oxysporum, C. lunata, Penicillium* spp., *Rhizopus* spp. and *Rhizoctonia bataticola* from seeds of ten chickpea varieties. Likewise, Kandhare (2014) identified 16 fungal species from chickpea seeds collected from different sources in India. Dawar *et al.* (2007) collected 14 samples of chickpea seeds from various regions of Pakistan and isolated fungi using agar plate, blotter paper and deep-freezing methods. They identified 21 fungal species of saprophytic and pathogenic fungi belonging to 13 fungal genera. Pre-dominant species were *M. phaseolina, Fusarium moniliforme* J. Sheld., *F. oxysporum, Rhizoctonia solani* Kuhn, *A. flavus* and *A. niger.* Four species namely *R. solani, Syncephalastrum* sp., *Absidia glauca* and *Trichoderma harzianum* were new reports on chickpea seeds from Pakistan. In general, 67 fungal species are known to be associated with chickpea seeds from various regions of the world (Nene *et. al*., 1996).

Seed germination ranged from 98–100% in surface sterilized and 88–96% in non-surface sterilized seeds. The difference in germination between treated and non-treated seeds was insignificant for all varieties except Thal (Fig. 1B). Radicle length ranged from 38–51 mm in treated and 30–37 mm in non-treated seeds. The difference in radicle length between treated and non-treated seeds was significant ($P = 0.05$) in all the four chickpea varieties (Fig. 1C). The correlation of germination and radicle length with total fungal incidence or with individual fungi ranged from negative to positive. However, generally these correlations were insignificant. The highest value of correlation coefficient (-0.95) was recorded between *P. italicum* and germination. The negative correlation with high coefficient value clearly indicates that germination in chickpea was mostly affected due to infestation of *P. italicum* during storage. Likewise, -0.72 value of correlation coefficient between *S. rolfsii* and radicle length revealed the remarkable effect of this fungal species on root growth of chickpea. *A. oryzae* incidence was also negatively correlated both with germination (-0.63) and radicle length (-0.57) as shown in Table 2. Sontakke and Hedawoo (2014) identified 13 fungal species from seeds of chickpea and found that seed germination was adversely affected by higher incidence of mycoflora. Toxins produced by fungi during storage deteriorate the stored products of the seeds (Afzal *et. al*., 1979), which result in low germination and radicle growth. In a similar study, Dawar *et al.* (2007) found that although most of the fungi were located on chickpea seeds, however, other parts such as axis of seed (radicle + plumule) and cotyledons were also attacked by fungi which may be responsible for reduced radicle length. Fungal species such as *A. niger* and *A. flavus* have been reported to damage the seeds during storage and also reduce seed germination percentage (Christensen, 1973).

The present study concludes that both pathogenic and stored grains fungi are associated with seeds of different chickpea varieties. Seeds of chickpea variety Noor were highly susceptible to fungal infestation. Germination and radicle length in chickpea were adversely affected generally by *P. italicum* and *S. rolfsii* infestations, respectively.

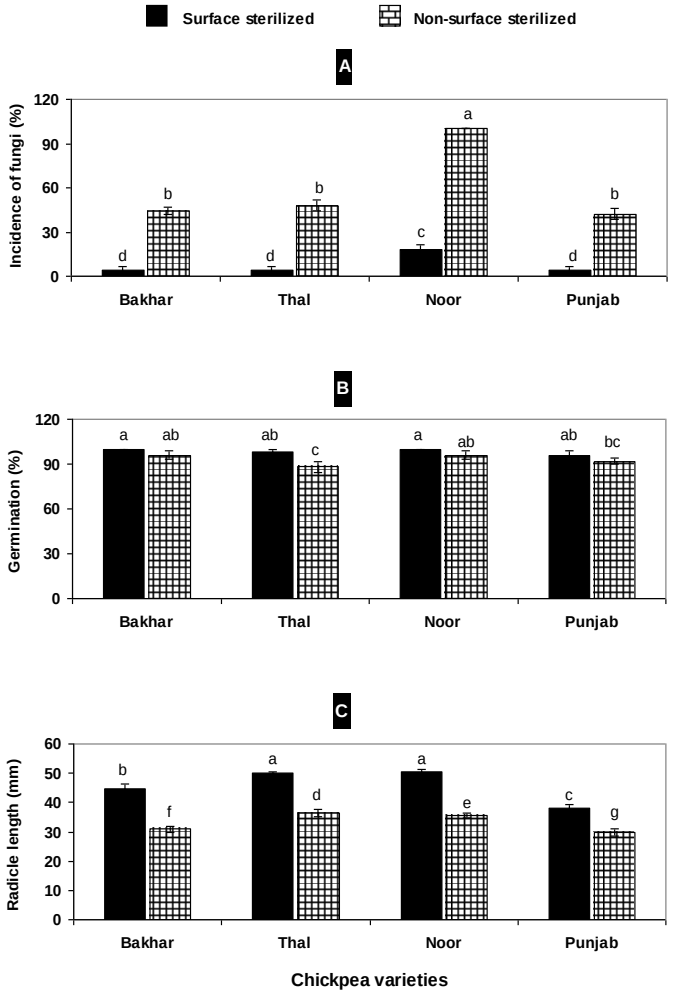

Fig. 1. Incidence of isolated mycoflora (A), germination percentage (B) and radicle length (C) in different chickpea varieties. Vertical bars show standard errors of means of five replicates. Values with different letters show significant difference ($p \leq 0.05$) as determined by LSD Test.

Table 1. Incidence of fungi isolated from surface sterilized (SS) and non-sterilized (NS) seeds of different chickpea varieties.

Chickpea variety	Treat-ments	Fungal incidence (%)								
		AA	AF	AN	AO	FO	MP	MS	PI	SR
Bhakar	SS	0 b	0 a	0 b	0 a	6 ab	0 b	0 b	0 b	0 b
	NS	2 b	2 a	0 b	0 a	15 a	0 b	4 b	2 b	4 b
Thal	SS	0 b	0 a	0 b	0 a	0 b	0 b	0 b	4 ab	0 b
	NS	0 b	2 a	2 b	4 a	6 ab	0 b	0 b	12 a	4 b
Noor	SS	6 ab	2 a	0 b	0 a	4 ab	8 a	0 b	4 ab	6 ab
	NS	15 a	6 a	20 a	2 a	0 b	4 ab	50 a	2 b	2 b
Punjab	SS	6 ab	0 a	2 b	0 a	4 ab	0 b	0 b	4 b	0 b
	NS	0 b	0 a	0 b	0 a	6 ab	6 ab	4 b	4 b	10 a

AA: *Alternaria alternata;* **AF:** *Aspergillus flavus;* **AN:** *Aspergillus niger;* **AP:** *Aspergillus oryzae;* ***FO*****:** *Fusarium oxysporum;* **MP:** *Macrophomina phaseolina;* **MS:** *Mucor* sp.; **PI:** *Penicillium italicum;* SR**:** *Sclerotium rolfsii*
In a column, values with different letters show significant difference as determined by LSD test at p = 0.05.

Table 2. Correlation of fungal flora (%) of chickpea with germination and radicle length in non-sterilized seeds.

	Germination (r)	Radicle length (r)
Total fungal flora	0.47	0.55
Alternaria alternata	0.61	0.42
Aspergillus flavus	0.48	0.64
Aspergillus niger	0.48	0.55
Aspergillus oryzae	-0.63	-0.57
Fusarium oxysporum	0.13	-0.57
Macrophomina phaseolina	0.17	-0.36
Mucor sp.	0.58	0.40
Penicillium italicum	-0.95	0.56
Sclerotium rolfsii	-0.30	-0.72

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(*Accepted for publication December 2018*)