Isolation and Identification of Seed-Borne Mycoflora Associated with Chickpea (*Cicer arietinum* L.)

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Abstract*—Chickpea (*Cicer arietinum*), annual plant of the pea family (Fabaceae), widely grown for its nutritious seeds. Chickpeas are an important food plant in India. The seeds are high in fibre and protein and are a good source of iron, phosphorus, and folic acid. Seed borne fungi affect chickpea crop leading to cause major loss in quality and quantity of the seed. Thus', in present study the seed borne mycoflora associated with chickpea crop were detected and isolated. During the present investigation seed samples of chickpea variety, VIJAY and JAKI-9218 were tested for the detection and identification of seed borne mycoflora by standard blotter paper method, agar plate method. The study aims at identifying seed borne fungi associated with chickpea seeds. Seed health testing is a pre-requisite for seed improvement, seed production, seed certification and trade in seed. Using blotter and agar plate methods as recommended by ISTA, the seed mycoflora of different chickpea seed samples were examined. Fungi were isolated from the seeds of different Chickpea varieties. Most dominant fungal species were *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum., Penicillium notatum., Rhizopus stolonifer, Alternaria alternata, Curvularia lunata, Rhizoctonia sp.

***Key words*— Seed borne fungi, Chickpea, agar plate, blotter paper**

I. INTRODUCTION

Chickpea, (*Cicer arietinum*), also called garbanzo bean or Bengal gram, annual plant of the pea family (Fabaceae), widely grown for its nutritious seeds. Chickpeas are an important food plant in India, Africa, and Central and South America. The seeds are high in fibre and protein and are a good source of iron, phosphorus, and folic acid. Similarly, chickpea, a staple crop in arid and semi-arid regions, is vulnerable to fungal infections caused by *Ascochyta rabiei*, *Fusarium oxysporum*, and *Rhizoctonia solani*, resulting in diseases such as Ascochyta blight, Fusarium wilt, and root rot (Walcott et al., 2006). These diseases can cause significant crop losses, particularly under favourable environmental conditions such as high humidity and soil moisture. Chickpea, a crucial crop in dry regions, also faces threats from fungi like *Ascochyta rabiei* and *Fusarium oxysporum*, resulting in diseases such as Ascochyta blight and Fusarium wilt. These diseases can lead to significant losses, especially in wet conditions.

Production: *Cicer arietinum* L. (chickpea) is the second most cultivated leguminous crop with 13.1 million tons per annum production, commonly grown on an estimated 13.5 million hectares of land in tropical, subtropical, temperate and semi-arid areas, worldwide. India is amongst the chief producers of chickpea, having 8.63 mha area under cultivation, 7.85 mt annum −1 production with an average yield of 900 kg per hectare (CIME, 2010; FAOSTAT, 2015; Muehlbauer and Sarker, 2017).

Economic importance: It constitutes 20-30% protein, 40-59% carbohydrate, 3% fibre, 3-6% oil, 4% ash, and is a good source of absorbable ions like Ca, P, Mg, Fe, K and essential B vitamins (Ibrikci et al., 2003) Green pods used are used as vegetable. Haulm is used as fodder, Excellent green intensive crop rotations. manure as it is easily decomposed when incorporated (Biomass has 1.5% N), Seed contains 25% protein, 1.15% fat and 62.6% carbohydrate, sprouted seeds which are rich in vitamins are consumed as salad, Flour is used in cakes. Starch is used in making noodles, being short duration fits well in many.

II. MATERIAL AND METHODS

Experimental location
Experimental location the experiment was conducted in the Department of Botany, Shivaji College Udgir, Maharashtra. Sources of experimental materials

Collection of seed samples

The method described by Neergaard (1973) has been adopted for the collection of seed samples. Accordingly, two random samples of seeds (250 gm each) were collected from local farmers and market places of Dharashiv and Beed districts of Maharashtra. Seed samples of Chickpea were collected for the isolation and identification of seed-borne fungi from Dharashiv and Beed district of Maharashtra farm.

Sr.no.	Local Name	English Name	Scientific Name	Family
1.	Chana	Chickpea	*Cicer arientinum* (L.)	Fabaceae / Leguminosae

Seed Varieties used in the present study are

Chickpea (*Cicer arientinum* L.)

i. *Cicer arientinum* (chickpea) Vijay

ii. *Cicer arientinum* (chickpea) Jaki-9218

During the course of studies, seed samples were separately collected and stored in pre-sterilized plastic containers without any treatment at laboratory conditions.

Detection of Seed Mycoflora

The seed mycoflora was isolated by using different methods such as Standard blotter paper method and Agar plate method as recommended by International Seed Testing Association ISTA (1966), De Tempe (1970), Neergaard (1973) and Agrawal (1976) Observations were recorded in percent incidence of seed borne fungi associated with unsterilized seeds. Fungi appeared on seed were isolated in pure culture for identification and for further study. Two different methods of isolation techniques were used for assessment of seed mycoflora.

Standard blotter paper method and Agar plate method as described by the International Seed Testing Association (ISTA) 1996, was used for the isolation of the seed-borne fungi associated with the seed samples.

i) Standard blotter paper method

This is the very simple, most convenient and efficient of all the incubation methods. Doyer (1938) was first to adopt blotter paper method in seed health management. In the blotter paper method, pair of sterile white blotter papers of 8.5cm diameter was soaked in sterile distilled water and were placed in pre-sterilized petri plates of 90mm diameter. Ten seeds per petri plates, in order to isolate only internal seed mycoflora, were surface sterilized for 2 minutes with 1% mercuric chloride solution followed by three subsequent washings in sterilized distilled water to remove mercuric chloride from seed and non-surface sterilized, were placed at equal distance on three layers of properly moistened sterilized blotters. These plates were incubated at a temperature at 27°C for 12 hrs in alternating cycles of light and darkness. The seeds were examined regularly for the growth of fungi over the seed. and fungal growth on seeds were observed after 7 days incubation. Chaudhari and Sharma (2015)

ii) Agar plate method

In Northern Ireland, Muskett and Malone (1941) first used this method for seed health management. Pre-sterilized Petri plate were poured with 20 ml of autoclaved Potato Dextrose Agar (PDA). On cooling the medium, ten seeds per plate of the sample to be studied were equidistantly placed aseptically. Incubation and other details of the study were same as described in blotter test method.

iii) Examination of incubated seeds

Sampling for identification of fungi was done at seventh days. The Petri dishes were brought to fungi. The compound microscope was used to determine the type of fungus in each plate. The seed-borne fungi were identified using identification keys and cross-checked for each seed plates to identify the type of fungus growing on each seed. (Mathur and Kongsdal, 2003) and pictoral atlas of soil and seed fungi. (Watanabe, 2002).

After seven days of incubation, fungal species found growing on the surface of seeds, were Identified and their percentage frequency (PF) of occurrence of fungal was calculated by applying the following formula:

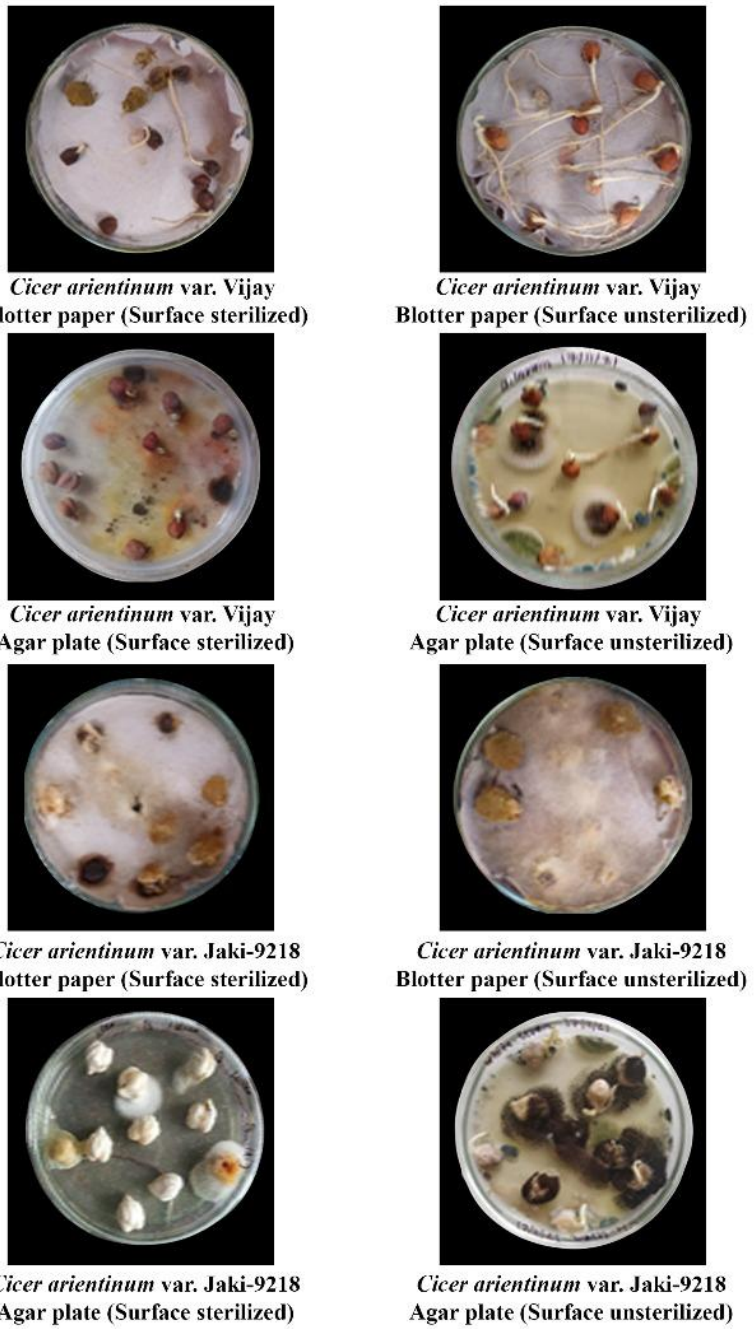
The percentage frequency (PF) of occurrence was calculated using the formula:

PF = (No. of seeds on which fungus appear / Total number of seeds) X 100. Chaudhari and Sharma (2015)

III. RESULTS

Photoplate 2

Seedborne fungi isolated from Chickpea by blotter paper and agar plate method



Cicer arientinum var. Vijay
Blotter paper (Surface sterilized)

Cicer arientinum var. Vijay
Blotter paper (Surface unsterilized)

Cicer arientinum var. Vijay
Agar plate (Surface sterilized)

Cicer arientinum var. Vijay
Agar plate (Surface unsterilized)

Cicer arientinum var. Jaki-9218
Blotter paper (Surface sterilized)

Cicer arientinum var. Jaki-9218
Blotter paper (Surface unsterilized)

Cicer arientinum var. Jaki-9218
Agar plate (Surface sterilized)

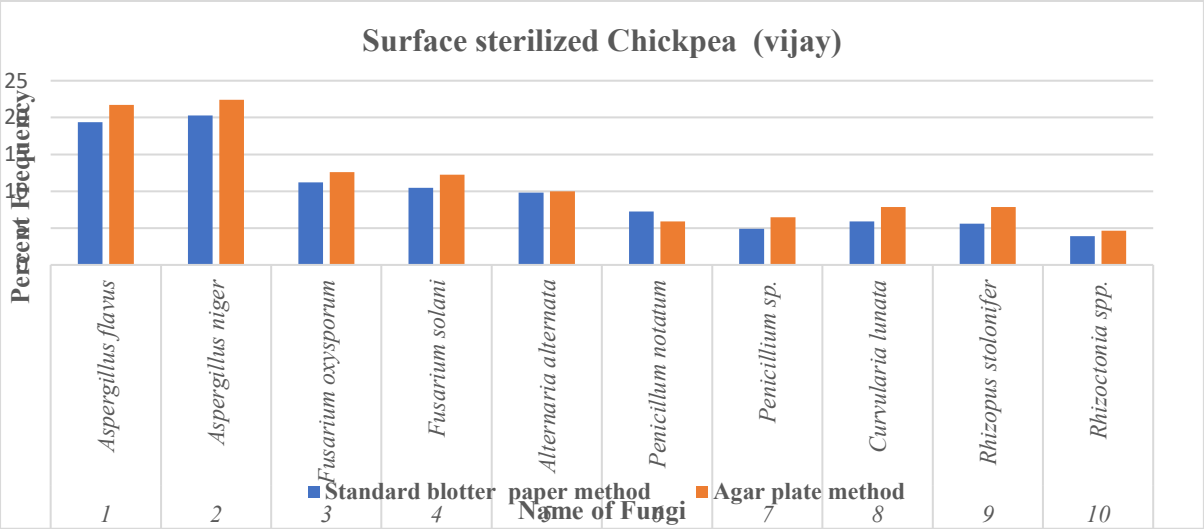
Cicer arientinum var. Jaki-9218
Agar plate (Surface unsterilized)

Fungi associated with seeds of *Cicer arientinum* (chickpea) Vijay:
The study analysed the frequency of mycoflora isolated from surface-sterilized and unsterilized chickpea seeds using two methods: the standard blotter paper method and the agar plate method.

Surface sterilized

Isolate no.	Name of Fungi	Percent Frequency of Mycoflora	Percent Frequency of Mycoflora
		Standard blotter paper method	Agar plate method
1.	*Aspergillus flavus*	19.38	21.7
2.	*Aspergillus niger*	20.3	22.4
3.	*Fusarium oxysporum*	11.2	12.6
4.	*Fusarium solani*	10.46	12.23
5.	*Alternaria alternata*	9.80	10
6.	*Penicillum notatum*	7.24	5.92
7.	*Penicillium sp.*	4.89	6.45
8.	*Curvularia lunata*	5.89	7.86
9.	*Rhizopus stolonifer*	5.58	7.84
10.	*Rhizoctonia spp.*	3.92	4.62

Table I. Percentage frequency of seed-borne fungi associated with naturally infected pigeon pea seeds

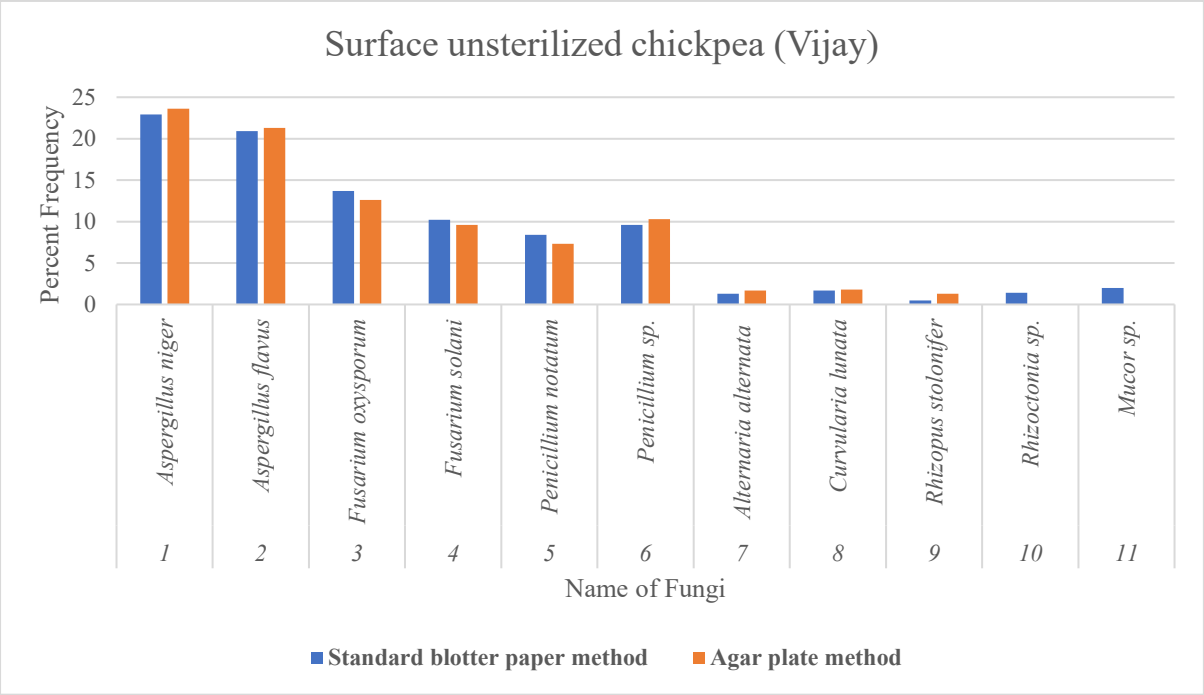


Surface Unsterilized:

Isolate no.	Name of Fungi	Percent Frequency of Mycoflora	Percent Frequency of Mycoflora
		Standard blotter paper method	Agar plate method
1.	*Aspergillus niger*	22.92	23.6
2.	*Aspergillus flavus*	20.9	21.3
3.	*Fusarium oxysporum*	13.7	12.6

4.	*Fusarium solani*	10.2	9.6
5.	*Penicillium notatum*	8.4	7.3
6.	*Penicillium sp.*	9.6	10.3
7.	*Alternaria alternata*	1.3	1.7
8.	*Curvularia lunata*	1.7	1.8
9.	*Rhizopus stolonifer*	0.5	1.3
10.	*Rhizoctonia sp.*	1.4	0
11.	*Mucor sp.*	2	0

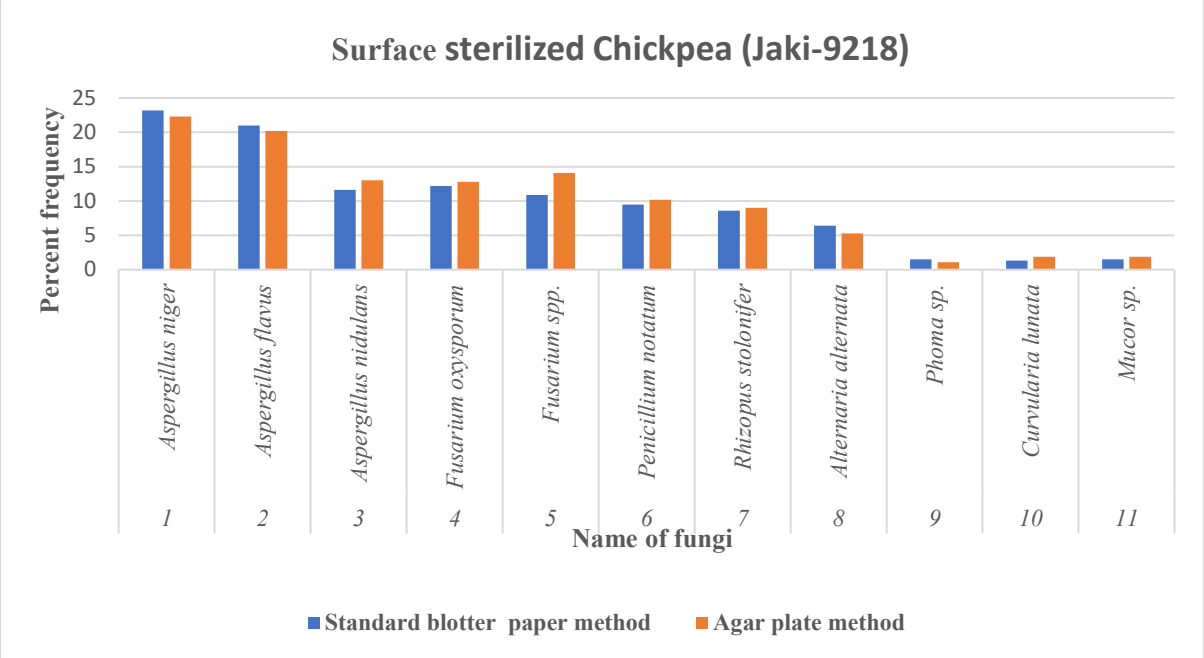
Table II. Percentage frequency of seed-borne fungi associated with naturally infected pigeon pea



Fungi associated with seeds of *Cicer arientinum* (chickpea) Jaki-9218:
Surface sterilized:

Isolate no.	Name of Fungi	Percent Frequency of Mycoflora	Percent Frequency of Mycoflora
		Standard blotter paper method	Agar plate method
1.	*Aspergillus niger*	23.2	22.3
2.	*Aspergillus flavus*	21.0	20.2
3.	*Aspergillus nidulans*	11.6	13
4.	*Fusarium oxysporum*	12.2	12.8
5.	*Fusarium spp.*	10.9	14.1
6.	*Penicillium notatum*	9.5	10.2
7.	*Rhizopus stolonifer*	8.6	9.0
8.	*Alternaria alternata*	6.4	5.3
9.	*Phoma sp.*	1.5	1.1
10.	*Curvularia lunata*	1.3	1.9
11.	*Mucor sp.*	1.5	1.9

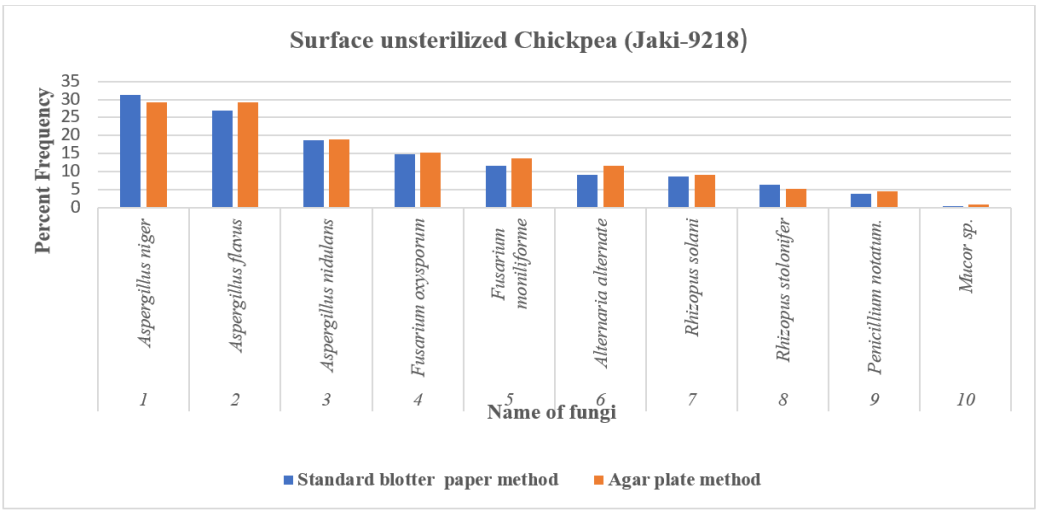
Table III. Percentage frequency of seed-borne fungi associated with naturally infected pigeon pea seeds



Surface Unsterilized:

Isolate no.	Name of Fungi	Percent Frequency of Mycoflora	Percent Frequency of Mycoflora
		Standard blotter paper method	Agar plate method
1.	*Aspergillus niger*	31.2	29.3
2.	*Aspergillus flavus*	26.9	29.2
3.	*Aspergillus nidulans*	18.7	19
4.	*Fusarium oxysporum*	14.8	15.3
5.	*Fusarium moniliforme*	11.5	13.7
6.	*Alternaria alternate*	9.2	11.5
7.	*Rhizopus solani*	8.6	9.0
8.	*Rhizopus stolonifer*	6.4	5.3
9.	*Penicillium notatum.*	3.73	4.54
10.	*Mucor sp.*	0.4	0.8

Table IV. Percentage frequency of seed-borne fungi associated with naturally infected pigeon pea seeds



IV. RESULTS AND DISCUSSION

The isolation of seed-borne mycoflora from *Cicer arietinum* (chickpea) variety Vijay was carried out using both surface-sterilized and surface-unsterilized seeds, and the results are presented in Tables 1 and 2. From the surface-sterilized seeds (Table 1), ten fungal species representing eight genera were isolated using both the blotter paper and agar plate methods. The blotter paper method revealed the presence of *Aspergillus niger* (20.3%) and *Aspergillus flavus* (19.38%) as dominant fungi, followed by *Fusarium oxysporum* (11.2%), *Fusarium solani* (10.46%), *Alternaria alternata* (9.80%), *Penicillium notatum* (7.24%), *Curvularia lunata* (5.89%), *Penicillium* sp. (4.89%), *Rhizopus stolonifer* (3.92%), and *Rhizoctonia* sp. (3.92%).

Using the agar plate method, a similar fungal spectrum was observed, with slightly higher incidence of several species. The predominant fungi included *Aspergillus niger* (22.4%) and *Aspergillus flavus* (21.7%), followed by *Fusarium oxysporum* (12.6%), *Fusarium solani* (12.23%), *Alternaria alternata* (10.0%), *Curvularia lunata* (7.86%), *Rhizopus stolonifer* (7.84%), *Penicillium* sp. (6.45%), *Penicillium notatum* (5.92%), and *Rhizoctonia* sp. (4.62%).

In the surface-unsterilized seeds of chickpea variety Vijay (Table 2), a total of eleven fungal species belonging to seven genera were isolated by both methods. The blotter paper method recorded higher frequencies of *Aspergillus niger* (22.92%) and *Aspergillus flavus* (20.9%), followed by *Fusarium solani* (13.7%), *Fusarium oxysporum* (10.2%), *Penicillium* sp. (9.6%), *Penicillium notatum* (8.4%), *Curvularia lunata* (1.7%), *Alternaria alternata* (1.3%), *Rhizoctonia* sp. (1.4%), *Rhizopus stolonifer* (0.5%), and *Mucor* sp. (2.0%).

Similarly, the agar plate method showed predominance of *Aspergillus niger* (23.6%) and *Aspergillus flavus* (21.3%), followed by *Fusarium solani* (12.7%), *Fusarium oxysporum* (9.6%), *Penicillium* sp. (10.3%), *Penicillium notatum* (7.3%), *Curvularia lunata* (1.8%), *Alternaria alternata* (1.7%), and *Rhizopus stolonifer* (1.3%).

For *Cicer arietinum* (chickpea) variety JAKI-9218, surface-sterilized seeds (Table 3) revealed the presence of eleven fungal species belonging to eight genera. The blotter paper method showed *Aspergillus niger* (23.2%) and *Aspergillus flavus* (20.2%) as dominant fungi, followed by *Fusarium oxysporum* (12.2%), *Aspergillus nidulans* (11.6%), *Fusarium* spp. (10.9%), *Penicillium notatum* (9.5%), *Rhizopus stolonifer* (8.6%), *Alternaria alternata* (6.4%), *Curvularia lunata* (1.3%), *Phoma* sp. (1.5%), and *Mucor* sp. (1.5%).

The agar plate method also detected a similar fungal composition, with higher incidence of *Aspergillus niger* (22.3%), *Aspergillus flavus* (20.2%), *Fusarium* spp. (14.1%), *Aspergillus nidulans* (13.0%), *Fusarium oxysporum* (12.8%), *Penicillium notatum* (10.2%), *Rhizopus stolonifer* (9.0%), *Alternaria alternata* (5.3%), *Curvularia lunata* (1.9%), *Phoma* sp. (1.1%), and *Mucor* sp. (1.9%).

Cicer arientinum (chickpea) Jaki-9218 :
In the chickpea variety *Cicer arietinum* (JAKI-9218), surface-unsterilized seeds (Table 4) revealed the presence of ten fungal species representing nine genera when examined using the blotter paper method. The predominant fungi detected were *Aspergillus niger* (31.2%) and *Aspergillus flavus* (26.9%), followed by *Aspergillus nidulans* (18.7%), *Fusarium oxysporum* (14.8%), *Fusarium moniliforme* (11.5%), *Alternaria alternata* (9.2%), *Rhizopus solani* (8.6%), and *Rhizopus stolonifer* (6.4%). Lower frequencies were recorded for *Penicillium notatum* (3.73%) and *Mucor* sp. (0.4%).

Similarly, the agar plate method detected comparable fungal diversity, with slightly higher incidence levels for several species. The dominant fungi included *Aspergillus niger* (29.3%) and *Aspergillus flavus* (29.2%), followed by *Aspergillus nidulans* (19.0%), *Fusarium oxysporum* (15.3%), *Fusarium moniliforme* (13.7%), *Alternaria alternata* (11.5%), *Rhizopus solani* (9.0%), and *Rhizopus stolonifer* (5.3%). Minor occurrences were observed for *Penicillium notatum* (4.54%) and *Mucor* sp. (0.8%).

The present findings are in agreement with the observations of Trivedi et al. (2007), who reported the occurrence of diverse seed-borne fungi using both blotter and agar plate techniques, including species of *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, and *Trichoderma*. Similar fungal associations in chickpea seeds were also documented by Sontakke and Hedawoo (2014), who identified thirteen seed-borne fungal species with variable frequencies, among

which *Aspergillus* and *Fusarium* species were predominant.

Comparable results were reported by Amule (2019), who detected eight major fungal genera across thirty chickpea varieties using the standard blotter method, with *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, and *Rhizopus* spp. occurring most frequently. These observations are further supported by studies conducted by Parashar (2019), Nanda (2020), and Chaudhari (2017), who reported similar seed-borne fungal profiles in chickpea.

The dominance of *Aspergillus flavus* and *Aspergillus niger* observed in the present study concurs with the findings of Patil et al. (2012) and Kandhare (2014), who reported high incidences of these fungi in chickpea and other legume seeds. Rathod et al. (2012) also recorded maximum occurrence of these species in various pulse crops using both blotter and agar plate methods. Furthermore, Ashwini and Giri (2014) observed a higher incidence of *Fusarium oxysporum* and *Aspergillus flavus* in green gram seeds, with minimal occurrence of *Curvularia lunata*. The association of seed-borne mycoflora recorded in this investigation is also consistent with the findings of Singh et al. (2014), Patil et al. (2012), and Chougule (2015), who reported a similar dominance of *Aspergillus*, *Fusarium*, and *Alternaria* species in chickpea seeds. Recent studies by Neme (2023), Srinivas (2017), and Vasava (2017) further corroborate these results, emphasizing the widespread prevalence of seed-borne fungi in chickpea and their potential impact on seed viability and crop health.

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