

Original Research Article

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Study to Detect Seed Borne Mycoflora Associated with Chickpea (*Cicer arietinum* L.) Seeds

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ABSTRACT

Madhya Pradesh is the largest producer of chickpea (*Cicer arietinum* L.) in India. A number of abiotic and biotic factors are responsible for its yield gaps of being below its potential. Therefore an study was conducted during two consecutive years 2015-16 and 2016-17 at Department of Plant Pathology, JNKVV, Jabalpur to detect seed borne mycoflora associated with Chickpea seeds by different methods of seed testing. During the study, total 30 varieties of chickpea viz., JG 130, JG 63, JG 16, JG 315, JG 11, JG 74, JG 322, JG 412, JG 226, JG 6, JG 14, JG 62, JG 218, Pragati, Uday, Vijay, Radhey, KJD 1168 (Alok), Avrodhi, JAKI 9218, BGD 72, BGD 128, BGD 1057, BGD 1058, Digvijay, Samrat, Vihar, GNG 1946, GNG 1845 and K 850 were collected and examined from different location of Madhya Pradesh by standard protocols of Standard blotter paper, 2,4-D blotter, Agar plate, Deep freeze, Paper towel and Seedling symptoms test method. The revealed that, the Standard Blotter Paper Method was found best method (110.0%) for testing of seed associated mycoflora followed by Agar Plate Method (103.2%), Deep Freeze Method (90.8%), Paper towel method (80.1%), Seedling symptoms test (75.3%) and 2, 4-D Method (56.4%). It was also concluded a total eight genera of fungi viz. *Fusarium oxysporum*, *Rhizoctonia bataticola*, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, *Rhizopus* spp. and *Penicillium* spp. were reported in different varieties of chickpea. Among them *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* spp. and *Fusarium oxysporum* were predominant to all seed samples.

Keywords

Chickpea, Seed borne myco-flora, Standard blotter paper, Agar plate method

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Introduction

In Indian Agriculture pulses play an important role not only from economical point of view but also due to their nutritional value. Among all the pulses chickpea is the most important one being rich sources of protein (21%), carbohydrates (61.57%) and vitamin C.

Chickpea producing states in India are Madhya Pradesh, Uttar Pradesh, Rajasthan, Maharashtra and Andhra Pradesh. Madhya Pradesh contributes 40.96 percent of the total production of chickpea in the country. In Madhya Pradesh it ranks 1st in both area (3.59 m. ha.) and production (4.6 m. tones) and the average productivity is estimated 1280 kg./ha.

Agri. Statistics at a glance, (2017-18). The number of diseases recorded on the chickpea has been increased and those which were formally of little importance have now become more significant.

There are more than 50 pathogens known to attack chickpea of which about 40 are of economic important. Many factors is involved in reducing chickpea seed quality but a combination of susceptibility to pathogenic fungi, environmental condition that favour disease development and field deterioration of seed. Moreover chickpea seeds are rich in protein contain and therefore easily infected by several field and storage fungi which not only affect seed quality but also emergence in field.

Detected several fungal species belonging to many genera from cultivars of chickpea seedborne fungi of chickpea have been also reported by various workers from time to time (Rathod *et al.*, 2012 and Narayan *et al.*, 2013).

Hence the present study on detection of seed-borne myco-flora of chickpea was conducted to know the seed mycoflora associated with the chickpea seeds from different locations of Madhya Pradesh.

Materials and Methods

Collection of seed samples of chickpea

Seeds of thirty chickpea cultivar were collected from farmer's fields of different agro climatic zones of Madhya Pradesh and All India Co-ordinated Research Project on Chickpea, Department of Plant Breeding & Genetics, JNKVV, Jabalpur (M.P.) viz., JG 130, JG 63, JG 16, JG 315, JG 11, JG 74, JG 322, JG 412, JG 226, JG 6, JG 14, JG 62, JG 218, Pragati, Uday, Vijay, Radhey, KJD 1168 (Alok), Avrodhi, JAKI 9218, BGD 72, BGD 128, BGD 1057, BGD 1058, Digvijay,

Samrat, Vihar, GNG 1946, GNG 1845 and K 850.

Blotter paper method

Standard blotter method was used for the detection of seed borne fungi of chickpea. The 100 seeds of each variety were sown on three layers of pre-soaked moist blotter paper having 9 cm diameter. In each plate 5 seed were arranged, 4 seeds in the outer ring and one in the center of petri plates.

Agar plate method

Agar plate method is preferred mostly in plant pathological studies as it provides nutrients rich substrate for development of mycelial growth and sporulation of pathogen on seed, particularly for slow growing fungi, PDA medium was autoclaved at 1.04 kg/cm² for 15 minute and warm medium approximately 15 ml per plate was poured. On solidification 100 seeds were plated, each plate containing 5 seeds.

Deep freeze method

This method allows better growth of certain fungi as the imbibed seeds on moist blotters are killed by deep freezing and the enclosed nutrients in seed are utilized by fungi. One hundred seeds were plated as in blotter method and incubated for 24hr at room temperature ($27 \pm 2^{\circ}\text{C}$) then the plated seeds were deep frozen at $- 20^{\circ}\text{C}$ for 24 hr.

2, 4 - D Method

2,4-D, is a herbicide retards seed germination and seedlings growth due to which the seeds are not displaced and the examination of fungi becomes easy, the blotter were soaked in 0.1 per cent 2,4-D suspension and then placed in plates. Two hundred seeds were incubated for seven days as in blotter method. The plated

seeds were then incubated at $27 \pm 20^\circ\text{C}$, under alternate cycle of 12 hr light and 12hr darkness for seven days by using two 40W white fluorescent tubes. After seven days of incubation, seeds were examined under stereoscopic microscope by using a magnification of 6X to 50X.

Research microscope was also used to confirm the identification of fungi based on morphological characters given in standard mycological books.

Rolled paper towel method

Randomly selected 100 seeds were placed on two layers of moist germination papers, which will be placed on a polythene paper, at ten seeds per row. These seeds will be covered with another moist germination paper and rolled carefully to avoid any excess pressure on seeds. The rolled towel papers will be kept in slanting position along the wall of laboratory tables and incubated at $26 \pm 2^\circ\text{C}$ for 7 days. The first count of germination was taken on 4th day and final count of 10th day. Morphologically normal seedlings were examined with stereo binocular microscope.

Seedling symptoms test

This method is developed by Khare, Mathur and Neergaard in 1977. 10 ml water agar is taken in each 16 mm diameter rimless test tube, sterilized and solidified with slight slant. One seed is sown in each test tube.

The tubes are placed for incubation at 20°C under artificial day light tubes, 12/12 h cycle. To retain moisture they are covered individually or in groups by a plastic sheet or aluminium foil which is removed when the seedlings have reached the cover. Seedlings are examined after 14 days for the typical symptoms of disease in the coleoptiles. The

symptoms can be easily studied being visible on roots as well as on green parts.

Results and Discussion

Table 1 showed that the result of comparative performance of various seed health detection methods employed in present study. The results revealed that, all the six seed health detection methods attempted were found to be efficient in detecting various myco-flora associated with the seeds of thirty chickpea varieties. However, higher percentages of the major eight seedborne fungi were evidenced by the seed health detection methods standard blotter paper method was found best method (110.0) for testing of seed associated fungi followed by Agar plate method (103.2 %), Deep freeze method (90.8 %), Paper towel method (80.1 %), Seedling symptoms test (75.3 %) and 2, 4 – D blotter method. These findings are inconformity with findings of Khan *et al.*, (1988), Arya *et al.*, (2004) and Warude *et al.*, (2016). Among all the method tested *Aspergillus niger* was observed maximum (30.6 %) in standard blotter paper followed by Agar plate (26.9 %), Deep freeze (24.9 %) and paper towel (24.5) was found predominant in all the thirty varieties of chickpea. *Aspergillus flavus* was observed maximum in standard blotter (23.3 %) followed by agar plate (20.8 %) while minimum was recorded in 2,4-D blotter (11.7%). In all eight mycoflora associated with chickpea seeds *Rhizopus* spp. was noticed maximum in standard blotter (22.4 %) while minimum was recorded in 2,4-D blotter (10.0 %) followed by paper towel (14.4%), seedling symptoms (16.8%). In agar plate detection method *Fusarium oxysporum* was observed maximum 20.0% followed by standard blotter 19.6% and Deep freeze 19.5%. Minimum association of *Rhizoctonia bataticola* was recorded in seedling symptoms method while maximum was observed in agar plate.

Table.1 Comparative evaluation of different detection method for association of myco-flora in chickpea seeds

S. No.	Fungi	Per cent myco-flora association on chickpea seed					
		Standard blotter paper method	2,4-D blotter method	Agar plate method	Deep freeze method	Paper towel method	Seedling symptoms test
1.	<i>Fusarium oxysporum</i>	19.6	12.0	20.0	19.5	16.6	14.1
2.	<i>Rhizoctonia bataticola</i>	1.8	1.7	2.8	1.6	1.8	1.5
3.	<i>Alternaria alternata</i>	5.1	4.0	4.4	3.5	3.8	3.2
4.	<i>Aspergillus flavus</i>	23.3	11.7	20.8	17.6	12.2	12.0
5.	<i>Aspergillus niger</i>	30.6	14.0	26.9	24.9	24.5	23.3
6.	<i>Curvularia lunata</i>	1.7	1.3	1.4	1.4	1.7	1.4
7.	<i>Rhizopus spp.</i>	22.4	10.0	18.7	17.6	14.4	16.8
8.	<i>Penicillium spp.</i>	5.5	1.7	8.2	4.7	5.1	3.0
Total fungi		110.0	56.4	103.2	90.8	80.1	75.3

In standard blotter *Alternaria alternata* (5.1%) was observed maximum. *Curvularia lunata* recorded ranged from 1.3 to 1.7 % in 2,4-D blotter and standard blotter, respectively. In agar plate method *Penicillium spp.* was observed maximum (8.2 %) followed by 5.5 % in standard blotter method. In present study, among six seed health detection methods attempted. The most efficient were Standard blotter paper and Agar plate methods.

These methods of seed health detection were also reported as most efficient and reliable for detection of various mycoflora associated with the seeds of soybean, sesame, sunflower, niger, chickpea, pigeonpea etc, by several earlier workers (Mandhare *et al.*, 2009). Sontakke and Hedawoo (2014) and Patil *et al.*, (2018).

It was concluded from the present study, amongst the six method used for testing of thirty chickpea varieties, the Standard Blotter Paper Method was found best method (110.0%) for testing of seed associated myco-flora followed by Agar Plate Method (103.2%), Deep Freeze Method (90.8%), Paper towel method (80.1%), Seedling

symptoms test (75.3%) and 2, 4-D Method (56.4%). It was also concluded a total eight genera of fungi *viz.* *Fusarium oxysporum*, *Rhizoctonia bataticola*, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, *Rhizopus spp.* and *Penicillium spp.* were reported in different varieties of chickpea.

Among them *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus spp.* and *Fusarium oxysporum* were predominant to all seed samples.

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