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## Seed health evaluation of Different varieties of Lentil by incubation methods

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### Abstract

Seeds of lentil harbour the seed borne mycoflora which adversely affect the germination and seedling vigour. Five varieties of lentil and one local variety were taken in the present investigation to assess the seed health by incubation methods. In this study, varying degree and types of mycoflora associated with lentil seeds were recorded. The mycoflora recorded were *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* sp., *Trichoderma* sp., *Nigrospora* sp., *Chaetomium globosum*, *Alternaria alternata*, *Curvularia lunata*, *Fusarium* sp., and *Rhizopus stolonifer*. In most of the methods, poor germination was observed in local variety, may be due to presence of seed associated mycoflora with higher frequencies whereas JL-3 variety has higher germination due to lower frequencies of detected mycoflora as compared to other varieties of lentil taken in the study.

**Keywords:** Lentil, seed borne mycoflora, seed health

### Introduction

The word lentil is derived from 'Latin' word "lens". Lentil (*Lens culinaris* Medik.) is the most ancient cultivated pulse crops among the legumes. Many common names are known as malka masoor (bold seeded), masuri (small seeded), masura and renuka. Masoor has been originated in East Mediterranean region, such as Asia Minor and Egypt. Lentil is most nutritious legume crop in India, mostly cultivated as rainfed crop during winter season after rice, maize, pearl millet or rainy fallow. Lentil is recognized one of the most nutritious legume crops ranking next to gram or chickpea amongst pulses. In lentil nutritive values are protein (24-26%), carbohydrate (57-60%), fat (1.3%), fibre (3.2%), phosphorus (300mg/100g), iron (Fe) (7mg/100g), vitamin-C (10-15mg/100g), calcium (Ca) (69mg/100g), vitamin-A (450 IU) and calorific value (343 Kcal/100g). In India, total lentil production during 2018-19, 1.53 mt (Anon., 2019)<sup>[7]</sup>.

Healthy seeds are the basis of healthy plants or crop, an important condition for good yield or outputs (Diaz *et al.* 1998)<sup>[13]</sup>. Diseases are main constraint in economically crop production as they cause heavy losses. The crop is affected by wide range of fungal diseases *viz.*, botrytis grey mould (*Botrytis fabae* and *B. cinerea*), lentil rust (*Uromyces viciae-fabae*), stemphylium blight (*Stemphylium botryosum*), anthracnose (*Colletotrichum truncatum*), fusarium wilt (*Fusarium oxysporum* f.sp. *lentis*) and collar rot (*Sclerotium rolfsii*). Masur (lentil) can be also contaminated by a wide range of viruses but mostly affect on yield is not as major as that caused by fungal microbes or pathogens. Masur (lentil) yellow disease caused by (BLRV) bean leaf roll virus, (BWYV) beet western yellows virus or (SCRLV) subterranean clover red leaf virus and (PSdMV) pea seed borne mosaic (Taylor *et al.*, 2007)<sup>[51]</sup>. Mostly fungal flora associated with seeds at the stage of harvesting, transporting, processing and under storage bring about certain undesirable changes, making them unfit for human consumption and sowing purpose. Presence or absence of seed borne pathogens on the seed coat or surface is significant aspect that determine the good quality of seed.

Till date, seed health evaluation aspects like mycoflora associated, their effect on germination of seedling vigour index and management not studied well and documented for different lentil varieties of Chhattisgarh. Therefore, an attempt is taken in the present investigation entitled seed health evaluation of different varieties of lentil (*Lens culinaris* M.) by incubation methods.

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## Methods and Materials

Unless and otherwise mentioned for each experiment, In each plates, ten seeds were placed on moist blotter paper in such a way that nine seeds formed the periphery of petri plate and one at the centre of petri plate. For each seed samples, 20 replicated plates were maintained (total of 200 seeds tested for each seed samples). Incubated the seeded plates at  $25\pm 1^\circ\text{C}$  for seven days in alternating cycles of 12 hours darkness and 12 hours light in NUV. Observations were recorded as explained earlier. All seeds of the periphery of plate were examined first, then finally seed in the centre of petri plate and expressed in percentage of seed associated mycoflora, individually. The organisms were observed under the stereobinocular microscope on seeds for their habit characters and confirmed under compound microscope.

The associated fungal flora were identified with the help of standard literature like Illustrated Genera of Imperfect Fungi (H.L. Barnett, 1962) [8], More Dematiaceous Hypomycetes (M.B. Ellis, 1976) and A Pictorial Guide to the Identification of Seed borne Fungi of Sorghum, Pearl Millet, Finger Millet, Chickpea, Pigeonpea and Groundnut (ICRISAT, 1978). Several methods of seed health testing have been developed for the detection of fungi associated with seeds. Some of the most suitable procedures have been recommended by the International Seed Testing Association (ISTA) (Anon., 1966). The following standard methods recommended were used in the present study for seed health evaluation of different varieties of pea.

1. Standard blotter method (ISTA, 1976) [20].
2. Agar plate method (Muskett and Malone, 1941) [35].
3. Roll paper towel method (Yaklich, 1985) [53].
4. Deep freeze method (Limonard, 1968) [32].

### 1. Standard blotter method

In standard blotter method, the fast growing fungi were better detected than the slow growing ones. In each sterilized interfitting plastic petri plates, two good quality sterilized blotter paper of the same diameter were kept and moist with sterilized distilled water. In each plates, ten seeds were placed on moist blotter paper in such a way that nine seeds formed the periphery of petri plate and one at the centre of petri plate. For each seed samples, 20 replicated plates were maintained (total of 200 seeds tested for each seed samples). Incubated the seeded plates at  $25\pm 1^\circ\text{C}$  for seven days in alternating cycles of 12 hours darkness and 12 hours light in NUV. Observations were recorded as explained earlier. All seeds of the periphery of plate were examined first, then finally seed in the centre of petri plate and expressed in percentage of seed associated mycoflora, individually.

**The frequency of the mycoflora was calculated by the following formula**

$$\frac{\text{No. of seeds containing a particular fungus}}{\text{Total seeds examined}} \times 100$$

### 2. Agar plate method

Potato dextrose agar (PDA) media (15-20 ml) was poured in each sterilized petri plates. A little amount of Streptomycin sulphate was added in the media at the time of pouring to avoid the bacterial contaminations. Seeds of each variety were surface sterilized with 1.0% NaOCl solution for 30 seconds and immediately washed twice with sterile distilled water

thoroughly to remove NaOCl solution adhered, if any. Seed were placed on the previously poured PDA medium in petri plate in such a manner that nine seeds in periphery and one at centre of petri plate. For each seed sample, 20 replicated plates were maintained and incubated at  $25\pm 1^\circ\text{C}$  under alternate cycles of 12 hours dark and 12 hours light in NUV. Observations were recorded as described earlier.

### 3. Roll paper towel method

The seeds (50) were placed on moist paper towel at equidistance and covered with the another moist paper towel and rolled carefully without disturbing already arranged seeds. Tie the towel with help of rubber band at both side ends. To avoid the water loss, polythene or wax coated paper used for wrapping the rolled paper towels containing seeds. Incubate it for 4 to 5 days at room temperature. Examine the normal and abnormal seedlings, cause of abnormalities, failure in germination and ungerminated seeds by naked eyes and presence of different mycoflora by stereoscopic binocular microscope. The observations were recorded for different categories were-

**Normal seedlings** - show the potential for continued growth and development into the satisfactory plants when grown in good quality soil and under favorable conditions of temperature, light and moisture.

#### Categories

1. **Intact seedlings:** Seedlings with all their essential structure, well developed, complete in proportion and healthy.
2. **Seedlings with slight defect:** Seedlings showing slight defect of their essential structure provided they show a satisfactory and balanced development comparable to that of intact seedlings of the same test.
3. **Seedlings with secondary infection:** Seedlings as described above but have been affected by fungi or bacteria.

**Abnormal seedlings:** Do not show the potential to develop into a plant when grown in good quality soil and under favorable condition of moisture, temperature and light.

#### Categories

1. **Damaged:** Seedlings with any of essential structure missing or badly and irreparably damaged and balance development cannot be expected.
2. **Deformed or unbalanced:** Seedlings with weak development or physiological disturbance or in which essential structures are deformed or out in proportion.
3. **Decayed:** Seedlings with any of their essential structure so diseased or decayed as a result of primary infection that normal development is prevented.

**Ungerminated seeds:** Did not germinated at the end of the test period.

1. **Hard seeds:** Seeds which have not absorbed water thus remain hard after end of the test period.
2. **Fresh seeds:** Seeds able to imbibe water but which failed to germinate under condition of the germination test remains clean and firm and have the potential to develop into the normal seedling.
3. **Dead seeds:** Seeds at the end of the test period are neither hard nor fresh, failed to produce a seedling; usually soft, discolored, frequently moldy.

**Other** – empty, embryo less seeds, intact damaged seeds.

#### 4. Deep freeze method

It was a modification of standard blotter method. The seeds were placed on blotter papers moistened with a solution containing 0.2% Streptopenicillin (to avoid bacterial contamination) and incubated for 24 hrs. under normal conditions in growth chamber. Plates were further incubated at  $10 \pm 1^\circ\text{C}$  for three days and then transferred to the deep freezer ( $-20^\circ\text{C}$ ) under complete darkness for 24 hrs. Plates were again incubated at  $20-25 \pm 1^\circ\text{C}$  for 5-7 days. Observations were recorded as described earlier.

### Result and Discussion

#### Standard blotter method

Seed lots of different varieties of lentil were evaluated for the seed associated mycoflora by using standard blotter method and data presented in the table 1 showed that the seed lot of local variety were found maximum frequency of mycoflora (129.99%) with minimum germination percentage (80.00%). Mycoflora were detected as *Aspergillus flavus* (30.00%), *Chaetomium globosum* (30.00%), *Fusarium* sp. (30.00%), *Rhizopus stolonifer* (23.33%), *A. niger* (13.33%) and *Alternaria alternata* (3.33%). This was followed by IPL-81 variety (116.65%) with germination percentage (90.00%) and detected mycoflora were as *Aspergillus flavus* (30.00%), *Fusarium* sp. (26.66%), *Rhizopus stolonifer* (23.33%), *Chaetomium globosum* (20.00%), *A. niger* (13.33%) and *Penicillium* sp. (3.33%).

In the seed lot of varieties L-4076, DPL-62, K-75 and JL-3, frequency of associated mycoflora were (106.64%), (103.32%), (103.30%) and (99.99%), respectively. In JL-3 variety, minimum frequency of mycoflora (99.99%) includes *Fusarium* sp. (26.66%), *Aspergillus flavus* (20.00%), *Rhizopus stolonifer* (20.00%), *Chaetomium globosum* (20.00%) and *Aspergillus niger* (13.33%) with maximum germination percentage (96.66%). In variety K-75, frequency of associated mycoflora namely *Aspergillus flavus* (26.66%), *Chaetomium globosum* (23.33%), *Rhizopus stolonifer* (16.66%), *Fusarium* sp. (16.66%), *A. niger* (10.00%), *Penicillium* sp. (6.66%) and *Alternaria alternata* (3.33%) with germination percentage (93.33%) were recorded. In variety DPL-62, frequency of associated seed borne mycoflora recorded as *Aspergillus flavus* (30.00%), *Fusarium* sp. (26.66%), *Rhizopus stolonifer* (20.00%), *Chaetomium globosum* (16.66%) and *Aspergillus niger* (10.00%) with germination percentage (96.66%). In variety L-4076, frequency of mycoflora (106.64%) includes *Fusarium* sp. (26.66%), *Chaetomium globosum* (23.33%), *Aspergillus flavus* (20.00%), *Rhizopus stolonifer* (16.66%), *Aspergillus niger* (13.33%), *Nigrospora* sp. (3.33%) and *Alternaria alternata* (3.33%). *Nigrospora* sp. found only in L-4076 variety.

The relative abundance of mycoflora *Aspergillus flavus* (142.40%) were found maximum from seed lot of lentil. Other predominated mycoflora were *Fusarium* sp. (139.53%), *Chaetomium globosum* (120.81%), *Rhizopus stolonifer* (109.06%), *A. niger* (66.87%), *Penicillium* sp. (9.30%), *Alternaria alternata* (8.90%) and *Nigrospora* sp. (3.12%).

In this method, local variety shows maximum frequency (129.99%) of mycoflora with minimum germination percentage while lowest frequency (99.99%) of mycoflora were observed in JL-3 variety among all the varieties of lentil taken in the study. Lentil varieties recorded *Aspergillus flavus*

(156.66%) in highest frequency and *Nigrospora* sp. (3.33%) in lowest frequency.

Present finding are in agreement of finding of El-Nagerabi and Elshafie (2000) [14], Allah and Hashem (2006) [4], Husasain *et al.* (2007) [19], Kayata and Agrawal (2010) [26] Hoque *et al.* (2014) [18], Mahal (2014) [33] and Lambat *et al.* (2015) [31] as they reported almost similar results for the seed associated mycoflora detected from seeds of lentil by using standard blotter paper method. Javaid *et al.* (2005) [21], Embaby and Abdel-Gail (2006) [15], Abdullah (2008) [1], Agrawal *et al.* (2011) [2], Rathod *et al.* (2012) [44], Ghangaoker and Kshirsagar (2013) [17], Narayan and Ayodhya (2013) [36], Embaby *et al.* (2013) [16], Pradhan (2017), Kumar *et al.* (2017) [28], Kawale *et al.* (2018) [25], Parashar *et al.* (2019) [38] and Kandhare (2020) [24] in legumes (green gram, black gram, chickpea and pigeonpea) reported the almost similar results for detected seed associated mycoflora by using the standard blotter paper method. Rahman *et al.* (1999) [43] in mungbean and black gram; Barua *et al.* (2007) [9], Patil *et al.* (2012) [37] and Sadhu (2014) [47] examined the different varieties of green gram by using standard blotter paper method and reported almost similar results. Sharma (2002) [48], Kumar *et al.* (2017) [28] and Kesharwani *et al.* (2018) [27] in pea; Raddy *et al.* (2006) [46], Pradhan *et al.* (2015) [39] and Chaudhari *et al.* (2016) [10] in pigeonpea; Dawar *et al.* (2007) [12], Razia and Pathak (2013) [45], Sontakke and Hedawoo (2014) [50], Chougule *et al.* (2015) [11], Warude (2016) [52], Amule *et al.* (2019) and Javid and Khan (2019) reported the results in different chickpea varieties and detected the seed associated mycoflora by using standard blotter paper method. Milad and Milad (2017) [34] in broad bean; Kumari and Saxena (2017) [29, 30] and Pradhan (2019) [40] in Indian bean; Zanjare *et al.* (2020) [54] in cowpea evaluated seed health by using blotter paper method and reported common seed associated mycoflora supports too findings of present study.

#### Agar plate method

Seeds of six varieties of lentil were evaluated for the associated seed borne mycoflora by agar plate method and the data presented in table 2. Maximum frequency of mycoflora were recorded from seeds of local variety (143.31%) which include *Aspergillus flavus* (30.00%), *Fusarium* sp. (30.00%), *Rhizopus stolonifer* (26.66%), *Chaetomium globosum* (23.33%), *A. niger* (20.00%), *Penicillium* sp. (6.66%) and *Alternaria alternata* (6.66%) with minimum germination percentage (70%) followed by IPL-81 seed lot mycoflora (139.99%) with germination percentage (76.66%). Frequencies of mycoflora recorded from the seeds of other varieties of lentil L-4076 (133.31%), K-75 (129.98%), DPL-62 (126.64%) and least in JL-3 (109.98%) with varying germination percentage as 76.66, 80.00, 83.33 and 86.66, respectively.

Different mycoflora were detected from lentil varieties by this method, in which *Aspergillus flavus* (169.98%) was recorded maximum and it was most frequent in local variety, L-4076 and IPL-81 (30.00%). This was followed by *Rhizopus stolonifer* (166.65%), *Fusarium* sp. (163.32%), *Chaetomium globosum* (129.97%), *A. niger* (103.31%), *Curvularia lunata* (36.66%), *Penicillium* sp. (6.66%) and *Alternaria alternata* (6.66%) was found least in lentil varieties.

The relative abundance of *Aspergillus flavus* (130.67%) was found maximum from seed lot of lentil. Other predominated mycoflora were *Rhizopus stolonifer* (128.94%), *Fusarium* sp. (123.78%), *Chaetomium globosum* (99.26%), *A. niger*



(82.71%), *Curvularia lunata* (28.87%). *Penicillium* sp. (4.18%) and *Alternaria alternata* (4.18%) was found least in relative abundance in lentil varieties.

Kaiser (1992) [23], Saheb *et al.* (2016) and Parashar *et al.* (2019) [38] in legumes (chickpea, mung, pigeonpea and lentil) detected and identified seed associated mycoflora by using agar plate method. Rathod *et al.* (2012) [44], Al- Tarjuman and Ramdan (2017) [5] and Kawale *et al.* (2018) [25] in different legume seeds (Pea, black gram, green gram and pigeonpea) recorded common seed borne mycoflora. Ali *et al.* (2010) [3], Sadhu (2014) [47] and Pradhan (2017) in mungbean seeds; Patil *et al.* (2012) [37], Pradhan *et al.* (2015) [39] and Chaudhari *et al.* (2016) [10] detected seed associated mycoflora in different varieties of pigeonpea. Sontakke and Hedawoo (2014) [50], Trivedi and Rathi (2015), Warude *et al.* (2010) and Amule *et al.* (2019) tested various varieties of chickpea and reported almost similar results for seed associated mycoflora. Kumar *et al.* (2017) [28] and Kesharwani *et al.* (2018) [27] in pea varieties; Kumari and Saxena (2017) [29, 30] in horsegram; Pradhan (2017) in mungbean; Kumari and Saxena (2017) and Pradhan (2019) [40] in Indian bean and Zanjare *et al.* (2020) [54] in cowpea reported seed borne mycoflora when evaluated by agar plate method. Findings of present study is in corroboration with the findings of above researchers as for as seed borne mycoflora is concern.

#### Rolled paper towel method

Seed lot of six varieties of lentil were examined for associated seed borne mycoflora in varying frequency with normal seedling, abnormal seedling and ungerminated seeds by roll paper towel method. It was observed that presence of mycoflora may be the cause of abnormalities and failure in seed germination. In this method, mycoflora were detected associated with seed and seedling of different lentil varieties. Maximum frequency of mycoflora were observed from local variety (110.00%) and mycoflora detected were *Aspergillus flavus* (26.00%), *Fusarium* sp. (24.00%), *Rhizopus stolonifer* and *Penicillium* sp. (18.00%), *Chaetomium globosum* (12.00%), *Aspergillus niger* and *Alternaria alternata* (6.00%) with minimum germination percentage (80.00%) followed by frequency of mycoflora in varieties IPL-81 (106.00%), K-75 (98.00%), DPL-62 (92.00%), L-4076 (92.00%) and JL-3 (82.00%) and germination percentage recorded in all these different lentil varieties were 80.00, 86.00, 90.00, 88.00 and 92.00, respectively.

In seeds of IPL-81 variety, mycoflora were recorded as *Aspergillus flavus* and *Fusarium* sp. (24.00%), *Rhizopus stolonifer* (18.00%), *Chaetomium globosum* (16.00%), *T. viride* (14.00%), *Aspergillus niger* (6.00%) and *Penicillium* sp. (4.00%). In K-75 variety, associated mycoflora were *Fusarium* sp. (24.00%), *Aspergillus flavus* and *Rhizopus stolonifer* (16.00%), *Trichoderma viride* (14.00%), *Aspergillus niger* and *Chaetomium globosum* (10.00%) and *Penicillium* sp. (8.00%). Mycoflora detected in DPL-62 variety were *Fusarium* sp. (20.00%), *Aspergillus flavus* (16.00%), *Rhizopus stolonifer* (14.00%), *Trichoderma viride* (14.00%), *Chaetomium globosum* and *Aspergillus niger* (10.00%) and *Penicillium* sp. (8.00%). In L-4076 variety, mycoflora detected were *Aspergillus flavus* and *Fusarium* sp. (24.00%), *Chaetomium globosum* (18.00%), *Rhizopus stolonifer* (10.00%), *Penicillium* sp. (8.00%), *Aspergillus niger* and *Alternaria alternata* (4.00%). In JL-3 variety, showed least frequency of mycoflora (82.00%) includes *Aspergillus flavus* and *Chaetomium globosum* (18.00%),

*Fusarium* sp. (18.00%), *Rhizopus stolonifer* and *Trichoderma viride* (12.00%) and *Aspergillus niger* (4.00%).

Relative abundance of mycoflora were *Fusarium* sp. (138.71%) followed by *Aspergillus flavus* (128.03%), *Rhizopus stolonifer* (90.40%), *Chaetomium globosum* (88.58%), *Trichoderma viride* (57.35%), *Penicillium* sp. (45.67%), *Aspergillus niger* (41.42%) and *Alternaria alternata* (9.80%).

Singh *et al.* (2014) recorded that the mycoflora and germination percentage of seeds (rice, wheat, pigeonpea and gram) and Sontake and Hedawoo (2014) in chickpea. Pradhan *et al.* (2015) [39] recorded commonly occurring seed borne mycoflora associated with seeds in pigeonpea by roll towel method namely as *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Penicillium* sp. and *Mucor* sp. Kesharwani *et al.* (2018) recorded seed associated fungal flora in different pea varieties as *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Trichoderma* sp., *Alternaria* sp., *Curvularia* sp., *Chaetomium* sp. and *Rhizopus* sp. by roll paper towel method. Amule *et al.* (2019) in chickpea; Pradhan and Lakpale (2020) [41] in different varieties of Indian bean seed observed different seed borne mycoflora by using roll paper towel method as *Aspergillus flavus*, *A. fumigatus*, *A. terreus*, *Curvularia lunata*, *Alternaria alternata* and *Fusarium* sp. Findings of present investigation are in agreement with the findings of earlier researchers that most of the common fungi in varying frequencies and their impact on germination were recorded.

#### 4. Deep freeze method

Seed lot of lentil varieties were tested for the associated seed borne mycoflora using deep freeze method and data presented in table 4. Frequency of mycoflora associated were maximum in local variety seed lot (89.98%) and mycoflora detected were *Aspergillus flavus* (26.66%), *Fusarium* sp. and *Chaetomium globosum* (23.33%), *Rhizopus stolonifer* (13.33%) and *Alternaria alternata* (3.33%) with minimum germination (73.33%) recorded. It was followed by L-4076 variety and IPL-81 (76.65%), K-75 (73.31%), DPL-62 (69.99%) and JL-3 (53.32%) and with the germination percentage of the lentil varieties were 76.66%, 80.00%, 76.66%, 83.33% and 90.00%, respectively.

Mycoflora observed in seed lot of L-4076 were *Fusarium* sp. (23.33%), *Aspergillus flavus* (20.00%), *Chaetomium globosum* (16.66%), *Rhizopus stolonifer* (13.33%) and *Alternaria alternata* (3.33%). In IPL-81 variety, seed borne mycoflora were *Aspergillus flavus* (26.66%), *Fusarium* sp. (23.33%), *Chaetomium globosum* (16.66%) and *A. niger* (10.00%). Mycoflora observed in K-75 variety were *Fusarium* sp. (26.66%), *Aspergillus flavus* (23.33%), *Chaetomium globosum* (13.33%), *Rhizopus stolonifer* (6.66%) and *A. niger* (3.33%). In variety DPL-62, recorded mycoflora were *Aspergillus flavus* (20.00%), *Fusarium* sp. (20.00%), *Chaetomium globosum* (16.66%) and *Rhizopus stolonifer* (13.33%). In variety JL-3, recorded mycoflora were *Fusarium* sp. (16.66%), *Aspergillus flavus* (13.33%), *Rhizopus stolonifer* (13.33%) and *Chaetomium globosum* (10.00%). In this method, relative abundance of different mycoflora such as *Fusarium* sp. (182.99%), *Aspergillus flavus* (175.90%), *Chaetomium globosum* (130.15%), *Rhizopus stolonifer* (85.34%), *A. niger* (17.26%) and *Alternaria alternata* (8.04%) were recorded.

Dawar *et al.* (2007) [12], Warude *et al.* (2016) [52] and Amule *et al.* (2019) Amule *et al.* (2019) also recorded seed associated mycoflora in chickpea seed by deep freeze method as

*Fusarium oxysporum*, *Rhizoctonia bataticola*, *Alternaria alternata*, *A. flavus*, *A. niger*, *Curvularia lunata*, *Rhizopus* sp. and *Penicillium* sp. Kesharwani *et al.* (2018)<sup>[27]</sup> examined different pea varieties by deep freeze method and detected seed associated mycoflora as *Aspergillus flavus*, *A. niger*, *Rhizopus* sp., *Alternaria* sp. and *Curvularia* sp. and Pradhan

(2019)<sup>[40]</sup> in Indian bean varieties by deep freeze method and detected associated mycoflora as *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Alternaria alternata*, *Curvularia lunata* and *Fusarium* sp. supports the findings of above the study due to occurrence of common seed borne mycoflora in various legumes.

**Table 1:** Detection of mycoflora associated with seeds of lentil varieties by standard blotter method

S. N.	Varieties	Germination (%)	Frequency of mycoflora associated (%) / Relative abundance (%)								Total frequency (%)
			<i>A. flavus</i>	<i>A. niger</i>	<i>Nigrospora</i> sp.	<i>Rhizopus stolonifer</i>	<i>Chaetomium globosum</i>	<i>Penicillium</i> sp.	<i>Fusarium</i> sp.	<i>Alternaria alternata</i>	
1	DPL-62	96.66	30.00 *(29.04)	10.00 (9.68)	-	20.00 (19.36)	16.66 (16.13)	-	26.66 (25.81)	-	103.32
2	IPL-81	90.00	30.00 (25.72)	13.33 (11.43)	-	23.33 (20.00)	20.00 (17.15)	3.33 (2.85)	26.66 (22.85)	-	116.65
3	JL-3	96.66	20.00 (20.00)	13.33 (13.33)	-	20.00 (20.00)	20.00 (20.00)	-	26.66 (26.66)	-	99.99
4	K-75	93.33	26.66 (25.81)	10.00 (9.68)	-	16.66 (16.13)	23.33 (22.58)	6.66 (6.45)	16.66 (16.13)	3.33 (3.22)	103.30
5	L-4076	90.00	20.00 (18.75)	13.33 (12.50)	3.33 (3.12)	16.66 (15.62)	23.33 (21.87)	-	26.66 (25.00)	3.33 (3.12)	106.64
6	Local variety	80.00	30.00 (23.08)	13.33 (10.25)	-	23.33 (17.95)	30.00 (23.08)	-	30.00 (23.08)	3.33 (2.56)	129.99
Total mycoflora (%)			156.66 (142.40)	73.32 (66.87)	3.33 (3.12)	119.98 (109.06)	133.32 (120.81)	9.99 (9.30)	153.30 (139.53)	9.99 (8.90)	

\* Relative abundance (%)

**Table 2:** Detection of mycoflora associated with seeds of lentil varieties by agar plate method

S. N.	Varieties	Germination (%)	Frequency of mycoflora associated (%) / Relative abundance (%)								Total frequency (%)
			<i>A. flavus</i>	<i>A. niger</i>	<i>Fusarium</i> sp.	<i>Chaetomium globosum</i>	<i>Penicillium</i> sp.	<i>Rhizopus stolonifer</i>	<i>Curvularia lunata</i>	<i>Alternaria alternata</i>	
1	DPL-62	83.33	26.66 *(21.05)	16.66 (13.15)	30.00 (23.69)	26.66 (21.05)	-	26.66 (21.05)	-	-	126.64
2	IPL-81	76.66	30.00 (21.43)	23.33 (21.21)	30.00 (21.43)	16.66 (11.90)	-	30.00 (21.43)	10.00 (7.14)	-	139.99
3	JL-3	86.66	26.66 (24.24)	10.00 (9.09)	16.66 (15.15)	16.66 (15.15)	-	30.00 (27.27)	10.00 (9.09)	-	109.98
4	K-75	80.00	26.66 (20.51)	16.66 (12.82)	30.00 (23.08)	20.00 (15.38)	-	30.00 (23.08)	6.66 (5.13)	-	129.98
5	L-4076	76.66	30.00 (22.51)	16.66 (12.49)	26.66 (19.50)	26.66 (19.50)	-	23.33 (17.50)	10.00 (7.51)	-	133.31
6	Local variety	70.00	30.00 (20.93)	20.00 (13.95)	30.00 (20.93)	23.33 (16.28)	6.66 (4.18)	26.66 (18.61)	-	6.66 (4.18)	143.31
Total mycoflora (%)			169.98 (130.67)	103.31 (82.71)	163.32 (123.78)	129.97 (99.26)	6.66 (4.18)	166.65 (128.94)	36.66 (28.87)	6.66 (4.18)	

\* Relative abundance (%)

**Table 3:** Detection of mycoflora with seeds of lentil varieties by roll paper towel method

S. N.	Varieties	Germination (%)	Frequency of mycoflora associated (%) / Relative abundance (%)								Total mycoflora (%)
			<i>A. flavus</i>	<i>A. niger</i>	<i>Chaetomium globosum</i>	<i>Alternaria alternata</i>	<i>Rhizopus stolonifer</i>	<i>Fusarium</i> sp.	<i>T. viride</i>	<i>Penicillium</i> sp.	
1	DPL-62	90.00	16.00 *(17.39)	10.00 (10.87)	10.00 (10.87)	-	14.00 (15.22)	20.00 (21.74)	14.00 (15.22)	8.00 (8.69)	92.00
2	IPL-81	80.00	24.00 (22.64)	6.00 (5.66)	16.00 (15.09)	-	18.00 (16.98)	24.00 (22.64)	14.00 (13.21)	4.00 (3.77)	106.00
3	JL-3	92.00	18.00 (21.95)	4.00 (4.88)	18.00 (21.95)	-	12.00 (14.64)	18.00 (21.95)	12.00 (14.64)	-	82.00
4	K-75	86.00	16.00 (16.33)	10.00 (10.21)	10.00 (10.21)	-	16.00 (16.33)	24.00 (24.48)	14.00 (14.28)	8.00 (8.16)	98.00
5	L-4076	88.00	24.00 (26.08)	4.00 (4.35)	18.00 (19.56)	4.00 (4.35)	10.00 (10.87)	24.00 (26.08)	-	8.00 (8.69)	92.00
6	Local variety	80.00	26.00 (23.64)	6.00 (5.45)	12.00 (10.90)	6.00 (5.45)	18.00 (16.36)	24.00 (21.82)	-	18.00 (16.36)	110.00
Total mycoflora (%)			124.00 (128.03)	40.00 (41.42)	84.00 (88.58)	10.00 (9.80)	88.00 (90.40)	134.00 (138.71)	54.00 (57.35)	46.00 (45.67)	

\* Relative abundance (%)

**Table 4:** Detection of mycoflora associated with seeds of lentil varieties by deep freeze method

S. N.	Varieties	Germination (%)	Frequency of mycoflora associated (%) / Relative abundance (%)						Total frequency (%)
			<i>A. flavus</i>	<i>A. niger</i>	<i>Fusarium</i> sp.	<i>Alternaria alternata</i>	<i>Chaetomium globosum</i>	<i>Rhizopus stolonifer</i>	
1	DPL-62	83.33	20.00 *(28.57)	-	20.00 (28.57)	-	16.66 (23.81)	13.33 (19.05)	69.99
2	IPL-81	80.00	26.66 (34.78)	10.00 (12.72)	23.33 (30.44)	-	16.66 (21.74)	-	76.65
3	JL-3	90.00	13.33 (25.00)	-	16.66 (31.25)	-	10.00 (18.75)	13.33 (25.00)	53.32
4	K-75	76.66	23.33 (31.83)	3.33 (4.54)	26.66 (36.36)	-	13.33 (18.18)	6.66 (9.08)	73.31
5	L-4076	76.66	20.00 (26.09)	-	23.33 (30.44)	3.33 (4.34)	16.66 (21.74)	13.33 (17.39)	76.65
6	Local variety	73.33	26.66 (29.63)	-	23.33 (25.93)	3.33 (3.70)	23.33 (25.93)	13.33 (14.82)	89.98
Total mycoflora (%)			129.98 (175.90)	13.33 (17.26)	133.31 (182.99)	6.66 (8.04)	96.64 (130.15)	59.98 (85.34)	

\* Relative abundance (%)

## Conclusion

The conclusion from the results of present study is as under

1. Seed health evaluation of six different varieties of lentil by using blotter paper, agar plate, rolled paper towel and deep freeze method reported common seed associated mycoflora.
2. Seed lots of six lentil varieties showed variation in frequency and types of seed borne mycoflora.

3. Mycoflora found associated with seeds of different lentil varieties were responsible for poor germination (Rolled paper towel method).

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