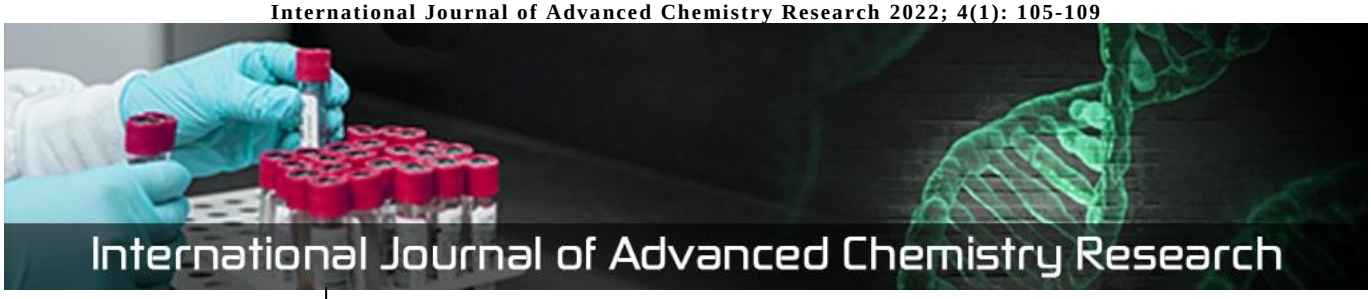

ISSN Print: 2664-6781
ISSN Online: 2664-679X
Impact Factor: RJIF 5.32
IJACR 2022; 4(1): 105-109
www.chemistryjournals.net
Received: 22-03-2022
Accepted: 23-04-2022

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Comparative efficacy of different incubation methods in detecting seed associated mycoflora of different varieties of lentil

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DOI: https://doi.org/10.33545/26646781.2022.v4.i1a.131

Abstract
A healthy or quality seed is a base of a healthy or good plant. Healthy seed refers to the presence or absence of various factors, among which the very important is seed associated fungi. Seed borne fungi cause reduction in healthy seed germination, vigour and viability. Lentil varieties were also found to be associated with several seed mycoflora. In the present study, comparative efficacy of four different incubation methods *viz*. standard blotter test, agar plate, paper roll towel and deep freeze method in detecting seed associated mycoflora in six varieties of lentil were examined and agar plate method was found best method for routine seed health evaluation.

Keywords: Seed health, lentil (*Lens culinaris* M), incubation method

Introduction
Lentil (*Lens culinaris* M) or masoor is the most ancient cultivated legume crops among the pulses. Lentil or masoor is most nutritious pulse crop in India, mostly cultivated as rainfed crop during Rabi season. Lentil plant is slender, semi-erect, bushy, annual, single stem/multi branched typically 20-45 cm long, plant produce many small shaped pods usually hold one or two seed each. Lentil is recognized one of the most nutritious legume crops ranking next to gram or chickpea amongst pulses. In lentil alimentary values are protein (24 to 26%), carbohydrate (57 to 60%), fat (1.3%), fibre (3.2%), phosphorus (300mg/100g), Fe (7mg/100g), vit-C (10 to 15mg/100g), Ca (69mg/100g), vit-A (450 IU) and cal. value (343 Kcal/100g) (Anonymous 2019 [2], Pulses revolution, Dept. of agriculture and farmer's welfare).
Infected seeds can often result in low germination quality and low seedling vigour, resulting in infected crop plants. Various field mycoflora associated with seeds and cause deterioration of quality of seed, viability and reduction of germination quality (Srivastava and Gupta, 1981) [24]. A large number of fungus was reported to be associated with the lentil seeds like *Aspergillus Niger*, *Aspergillus flavus*, *Rhizopus stolonifer Chaetomium globosum*, *Trichoderma viride* and *Cladosporium* sp. "Seed health determining the presence or absence of disease causing micro-organisms of various kind". Healthy seed is basic input in agriculture, a healthy or disease free seed leads to a healthy crop harvest. In the present investigation, different incubation methods were compared for their efficiency is detecting seed borne fungi associated with lentil seeds.

Materials and Methods
Unless and otherwise mentioned for each experiment, 400 seeds were used. In general, the petridishes with seeds were incubated at 22±1˚C under a 12 hour's dark and light cycle with NUV light for seven days. Observations were recorded seven days after seeding for the type of mycoflora associated and its frequency. The micro-organism were observed under the stereo binocular microscope on the seed for their habit characters and then confirmed under the compound microscope.
The associated mycoflora were identified with the help of standard literature like Illustrated Genera of Imperfect Fungi (H.L. Barnett, 1962) [4], More Dematiaceous Hypomycetes (M.B. Ellis, 1976) [7],

A Pictorial Guide to the Detection of Seed bone Fungi of Chickpea, Pigeonpea, Indian bean, Sorghum, Pearl Millet, Finger Millet and Groundnut (ICRISAT, 1978) [8] and FUSARIUM SPECIES, An Illustrated Manual for Identification Some methods of seed health testing have been evaluated for the identification of mycoflora associated with seeds. Some of the most suitable techniques have been suggested by the International Seed Testing Association (ISTA). The methods to detect micro-organism in or on the seed differ quite markedly depending on the location of the micro-organism and the mode of seed transmission (Neergaard, 1977) [16] and the specific group to which micro-organism belongs.

Incubation methods

- Standard blotter method
- Agar plate method (Muskett and Malone, 1941) [15]
- Roll paper towel method (Yaklich, 1985) [26]
- Deep freeze method (Limonard, 1968) [14]

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±1˚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

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

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 center of petri plate. For each seed sample, 20 replicated plates were maintained and incubated at 25±1˚C under alternate cycles of 12 hours dark and 12 hours light in NUV. Observations were recorded as described earlier.

Roll paper towel method

The seeds (50) were placed on moist paper towel at equidistance and covered with 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 unterminated 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**

a. **Intact seedlings:** Seedlings with all their essential structure, well developed, complete in proportion and healthy.
b. **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.
c. **Seedlings with secondary infection:** Seedlings as described above but have been affected by fungi or bacteria.
d. **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

- **I Damaged:** Seedlings with any of essential structure missing or badly and irreparably damaged and balance development cannot be expected.
- **Deformed or unbalanced:** Seedlings with weak development or physiological disturbance or in which essential structures are deformed or out in proportion.
- **Decayed:** Seedlings with any of their essential structure so diseased or decayed as a result of primary infection that normal development is prevented.
- **Unterminated seeds:** Did not germinated at the end of the test period.
- **Hard seeds:** Seeds which have not absorbed water thus remain hard after end of the test period.
- **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.
- **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.

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±1˚C for three days and then transferred to the deep freezer (-20 ˚C) under complete darkness for 24 hrs. Plates were again incubated at 20-25±1 ˚C for 5-7 days. Observations were recorded as described earlier.

Result and Discussion

Comparative efficacy of four incubation methods *viz.* standard blotter, agar plate, rolled paper towel and deep freeze methods in detecting seed borne mycoflora in six varieties of lentil were presented in table 4.2.6 (a). Among them, agar plate method was found to the best for routine seed health evaluation as it could detect (130.54%) mean frequency of mycoflora as compared to (109.98%) in standard blotter paper method, (96.67%) in roll paper towel method and (73.32%) in deep freeze method.

Per cent efficiency wise, agar plate method recorded maximum efficiency (78.04%) over deep freeze method followed by (35.04%) over roll paper towel method and (18.69%) over standard blotter paper method.

1. Efficiency of agar plate method over deep freeze method-

$$\frac{130.54 - 73.32}{73.32} \times 100 = 78.04\%$$

2. Efficiency of agar plate method over roll paper towel method-

$$\frac{130.54 - 96.67}{96.67} \times 100 = 35.04\%$$

3. Efficiency of agar plate method over standard blotter method-

$$\frac{130.54 - 109.98}{109.98} \times 100 = 18.69\%$$

Rathod *et al.* (2012) [22], Patil *et al.* (2012) [18], Chaudhari (2016) [5] in different legume crops and Kesharwani *et al.* (2018) [12] examined the comparative efficacy of different incubation methods in detecting seed borne mycoflora in different pea varieties, also reported that agar plate method was found to be the most efficient method for detection of seed associated mycoflora in seeds of different legumes, agreeing with the findings of present study. On the contrary, Dawar *et al.* (2007) [6], Razia and Pathak (2013) [21], Sontakke and Hedawoo (2014) [25], Pradhan *et al.* (2015) [19], Saheb *et al.* (2016), Parashar *et al*. in legume crops (gram, mung bean, arhar and lentil), Kumari and Saxena (2017), Amule *et al.* (2019), Pradhan (2019) [20] in Indian bean and Zanjare *et al.* (2020) [20] recorded that the standard blotter method was suitable for the detecting seed associated different mycoflora among all the incubation methods when employed in different legumes. Efficiency of mycoflora detecting incubation methods may be depends on the types of crop seeds and mycoflora harbour by the seeds, grain conditions, cropping seasons, crop handling and storage conditions.

Data presented in table-(a) for overall mycoflora detected by four incubation methods revealed that seeds of local variety harbor highest mean mycoflora (118.32%) followed by IPL-81 (109.82%), L-4076 (102.15%), K-75 (101.15%), DPL-62 (97.98%) and lowest in variety of JL-3 (86.32%).

Different mycoflora were detected by various incubation methods in varying frequencies given in table-(b). *Aspergillus flavus* was detected with maximum frequency (156.66%) followed by *Fusarium* sp. (153.30%) and *Chaetomium globosum* (133.32%) in standard blotter method. In agar plate method, highest frequency of *Aspergillus flavus* (169.98%) was recorded followed by *Rhizopus stolonifer* (166.65%) and *Fusarium* sp. (163.32%). In rolled paper towel method, highest frequency of *Fusarium* sp. (134.00%) and followed by *Aspergillus flavus* (124.00%) and *Rhizopus stolonifer* (88.00%) were recorded. *Fusarium* sp. (133.31%) was detected with maximum frequency followed by *Aspergillus flavus* (129.00%) and *Chaetomium globosum* (59.98%) in deep freeze method.

Total 10 number of mycoflora were recorded from all the lentil varieties taken in the study. Among them *Curvularia lunata* and *Trichoderma viride* were not detected in standard blotter method; *Nigrospora* sp. and *Trichoderma viride* in agar plate method; *Curvularia lunata* and *Nigrospora* sp. in roll paper towel method; *Curvularia lunata*, *Nigrospora* sp., *Penicillium* sp. and *Trichoderma viride* in deep freeze method were not detected.

Table (a): Comparative efficacy of different incubation methods in detecting seed borne mycoflora of different lentil varieties

S. No.	Varieties	Detection method				Mean Frequency (%)
		Frequency of mycoflora (%)				
		Standard blotter method	Agar plate method	Roll paper towel method	Deep freeze method	
1	DPL-62	103.32	126.64	92.00	69.99	97.98
2	IPL-81	116.65	139.99	106.00	76.65	109.82
3	JL-3	99.99	109.98	82.00	53.32	86.32
4	K-75	103.30	129.98	98.00	73.31	101.14
5	L-4076	106.64	133.31	92.00	76.65	102.15
6	Local variety	129.99	143.31	110.00	89.98	118.32
7	Total mycoflora	659.89	783.21	580.00	439.90	
8	Mean	109.98	130.54	96.67	73.32	

Table (b): Comparative efficacy of different incubation methods in detecting seed borne mycoflora of different lentil varieties

S. No.	Mycoflora	Detection method				Mean Frequency (%)
		Associated mycoflora (%)				
		Standard blotter method	Agar plate method	Roll paper towel Method	Deep freeze Method	
1	*A. Flavus*	156.66	169.98	124.00	129.00	144.91
2	*A. Niger*	73.32	103.31	40.00	13.33	57.49
3	*Alternaria Alternata*	9.99	6.66	10.00	6.66	8.33
4	*Curvularia Lunata*	-	36.66	-	-	9.16

5	*Chaetomium Globosum*	133.32	129.97	84.00	96.64	110.98
6	*Fusarium* sp.	153.30	163.32	134.00	133.31	145.98
7	*Nigrospora* sp.	3.33	-	-	-	0.83
8	*Penicillium* sp.	9.99	6.66	46.00	-	15.66
9	*Rhizopus stolonifer*	119.98	166.65	88.00	59.98	108.65
10	*Trichoderma viride*	-	-	54.00	-	13.50
11	Total mycoflora	659.89	783.21	580.00	438.92	
12	Mean	65.99	78.32	58.00	43.89	

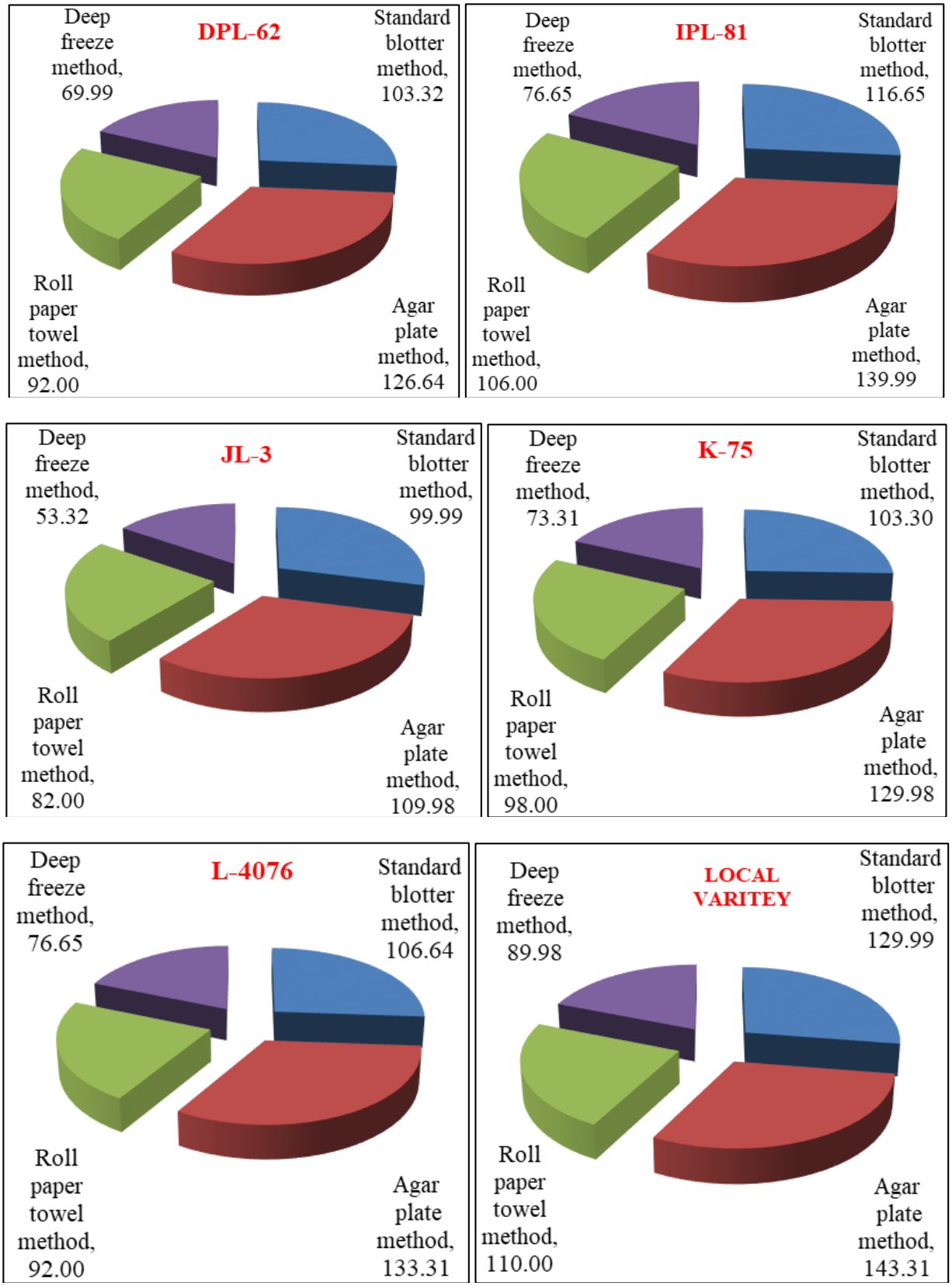

Fig 1: Comparative efficacy of different incubation methods in detection of Seed borne mycoflora in different lentil varieties

(a) Standard blotter paper method

(b) Agar plate method

(c) Roll paper towel method

Conclusion

Comparative efficacy of four incubation methods *viz*. standard blotter paper, agar plate, roll paper towel and deep freeze method in detecting seed associate fungi in six varieties of lentil were worked out. Among them, agar plate method was found to be very effective seed health evaluation of lentil varieties.

Reference

1. Amule R, Singh R, Gupta O, Raipuriya, Gupta PK. Study to detect seed borne mycoflora associated with chickpea (*Cicer aeritinum* L.) seeds. Int. J Curr. Microbiol. App. Sci. 2019;8(11):424-428.
2. Anonymous. Pulses Revolution: From food to nutritional security, 2018-19. Govt. of India, Ministry of Agriculture & Farmer's Welfare, Department of Agriculture, Cooperation & Farmers Welfare; c2019. p. 22-94.
3. Anonymous. Commodity Profile for Pulses. Directorate of Economics and Statistics, Department of Agriculture & Cooperative and Department of Commerce; c2019. p. 1-11.
4. Barnett HL. Illustrated genera of imperfect fungi. 2nd ed. Burgess Publishing Company, Minn; c1962.
5. Chaudhari AK, Sharma H, Jehani M, Sharma JK. Seed mycoflora associated with pigeonpea (*Cajanus cajan* (L.) Millsp.), their significance and the management. Journal of pure and applied microbiology. 2016 Mar 1;11(1):567-575.
6. Dawar S, Syed F, Ghaffar A. Sees borne fungi associated with chickpea in Pakistan. Pak. J Bot. 2007;39(2):637-643.
7. Ellis MB. More dematiaceous hypomycetes. CABI International, Wallingford, UK; c1976.
8. ICRISAT. A pictorial guide to the identification of seed borne fungi of sorghum, pearl millet, chickpea, pigeonpea and groundnut. International crops research institute for the semi-arid tropics. Info Bulletin; c1976. p. 34.
9. ISTA. Seed health testing. International rules for seed testing. Seed Sci. and Technol. 1966;4:31-34.
10. ISTA Rules. International rules for seed testing. Seed Sci. and Technol. 1985;13:307-502. ISTA; c1999.
11. ISTA. International Rules for Seed Testing. International Seed Testing Association, Switzerland; c2006.
12. Kesharwani A, Lakpale N, Khare N, Tiwari PK. Comparative efficacy of different incubation methods in detecting seed borne mycoflora. Res. J Agriculture Sciences. 2018;9(4):883-885.
13. Kumari DS, Saxena N. Seed borne mycoflora of *Macrotyloma uniflorum* L. (Horse gram). Int. J Curr. Microbiol. App. Sci. 2017;6(1):373-378.
14. Limonard T. A modified blotter test for seed health. Netherlands J Pl. Path. 1968;72:319-321.
15. Muskett AK, Malone JP. The alter method for the examination of flax seed for the presence of seed borne parasites. Am. Appl. Biol. 1941 Feb;28(1):8-13.
16. Neergaard P. Seed Pathology, The Macmillan Press Ltd., London. 1977;1:2.
17. Parashar R, Rizvi G, Sinha P. Seed mycoflora of some pulses collected from Bundelkhand region. J Pharmacognosy and Phytochemistry. 2019;8(3):1981-1985.
18. Patil DP, Pawar PV, Muley SM. Mycoflora associated with pigeon pea and chickpea. Int. Multidisciplinary Res. Journal. 2012;2(6):10-12.
19. Pradhan A, Lakpale N, Khare N. Effect of seed priming by seed-borne mycoflora on plant vigour of pigeonpea (*Cajanus cajan* (L.) Millsp.). An International Quarterly J. Environmental Sciences. 2015;7:403-406.
20. Pradhan K. Seed health evaluation of different varieties of Indian bean. M.Sc. (Ag.) Thesis submitted to Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.); c2019.
21. Razia KZ, Pathak N. Evaluation of seed infection of fungi in Chickpea. J Sci. and Tech. 2013;8(2):27-36.
22. Rathod LR, Jadhav MD, Mane SK, Muley SM, Deshmukh PS. Seed borne Mycoflora of legume seeds. Int. J Adv. Biotech. Res. 2012;3(1):530-532.
23. Sahab AF, Amin HA, Ziedan SH. Seed borne fungal pathogens associated with common Egyption seeds and their efficiency to produce saponin hydrolase enzyme. Int. J Chem Tech Res. 2016;9(11):299-306.
24. Shrivastva RN, Gupta JS. Seed mycoflora from Indian seed lots from *Cosmos bipinatus* and their control. Indian Phytopath. 1981;34(3):383-385.
25. Sontakke NR, Hedawoo GB. Mycoflora associated with seeds of chickpea. Int. J of Life Sci. 2014;(A2):27-30.
26. Yaklich RW. Rules for testing seeds. J Seed Tech. 1985;6(2).
27. Zanjare SR, Balgude YS, Zanjare SS, Suryawanshi AV, Shelar VR. Detection of seed borne myco-flora associated with cowpea (*Vigna unguiculata* L. Walp). Int. J Chem. Studies. 2020;8(1):1585-1587.