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Seed Health Evaluation of Different Varieties of Lentil by Physical Methods

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Abstract: **Seed health evaluation was attempted for seed lots of different varieties of lentil *viz*. DPL-62, IPL-81, JL-3, K-75, L-4076 and local variety from randomly selected village. Dry seed examination showed the status of healthy seeds, damaged seeds, shrunken seeds, small or under sized seeds, discoloured seeds, inert matter and other crop variety seeds in seed lots. Dry seed examination revealed that JL-3 variety showed the maximum purity as compare to other lentil varieties taken in the study and local variety were found as minimum pure among all the varieties. Washing test was also performed and maximum spore load was recorded from seed lot of local variety which include i.e. 20×10^2 which includes eight spores of *Rhizopus stolonifer*, three spores of *Aspergillus flavus*, two spores each of *Aspergillus niger, Chaetomium globosum*, *Trichoderma viride* and *Cladosporium* sp. and one spore of *Alternaria alternata*. While JL-3 and K-75 (13×10^2) variety showed the minimum spore load among all the lentil varieties taken in the study.**

***Keywords*— Seed health, lentil, physical method**

I. Introduction

Lentil (Lens culinaris M) or masoor is the most ancient cultivated pulse crops among the pulses/legumes. Lentil plant is mostly slender, semi-erect, bushy, leaves are small, compound, 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. Lentil or masoor is most nutritious pulse crop in India, mostly cultivated as rainfed crop during rabi season. It has potential to cover risks of dry land agriculture and used as cover crop. 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, Pulses revolution, Dept of agriculture and farmer's welfare).

Availability of healthy seed is the key for successful healthy crop cultivation. Infected seeds can often bring about poor germination and poor seedling vigour, resulting in damaged plant/crop. Presence or absence of seed varieties of nutritive values likes vitamins, proteins and carbohydrates in legume crop makes its responsible to infect by various seed associated myco-flora. These seed associated mycoflora responsible for pre and post crop losses during the crop growth stage, harvesting, transport, storage and processing, under unfavourable changes. Seed associated mycoflora play more important role in determining good quality of seed and viability of seed (Neergaard, 1977). Seeds are mostly associated with certain saprophytic/ parasitic pathogens which maintain in the seeds on the unfavourable condition. Some of the most suitable techniques have been suggested by the International Seed Testing Association (ISTA) (Anon., 1966). An attempt was made for lentil seed health evaluation by physical methods (dry seed examination and washing out test).

II. Materials and Methods

1. Dry seed examination

Inspection of dry seeds can be applied to detect seed associated mycoflora, when present in the seeds may cause change in seed shape and size or appearance of seed surface. First screened the seed sample (100 gm) by naked eyes, followed by magnifying lens and then examine under stereo-binocular microscope to record observations on the mixture of seeds, healthy, damaged seed, discoloured, plant parts, weed seeds, insert matter, other crop seeds, fraction such as soil, stones, sand, malformations, fungal bodies etc.

2. Washing test

Washing test was conducted to detected and identify the spores of mycoflora attached on the seed surface. Two gram of seed from working sample was taken with 10 ml of sterile distilled water in a test tube and with the help of a mechanical shaker, shake the test tubes for 10 minutes to remove the adhering parts of associated micro-organisms from the seeds. Suspended spores were concentrated by centrifuging at 3000 rpm for 15 to 20 minutes. The pallets used to make serial dilution of spore suspension and the suspension was discarded. For the serial dilution of six seed samples, arranged in 18 test tubes in a row on test tube stand and fill all with 9 ml of sterilized distilled water individually. Add 1 ml of stock spore suspension in each test tube containing 9 ml of distilled water to give 10-1 dilution of fungal spores suspension. Label it, mix thoroughly and then add 1 ml of 10-1 dilution to the next test tube containing 9 ml of distilled water to give 10-2 dilution in second dilution. These spore suspension spread on the PDA poured plates of each dilution in three replicated plates. These plates were incubated at 25±1˚C under a 12 hours dark and light cycle with NUV light for the 4-5 days. Observations were recorded for the identification of mycoflora with the help of microscope and indicated in terms of spore load counted by counting colonies of individual mycoflora.

III. Result and discussion

1. Dry seed examination

Dry seed examination showed the status of healthy seeds, damaged seeds, shrunken seeds, small or under sized seeds, discoloured seeds, inert matter and other crop variety seeds in seed lots. Lentil seed lots of various varieties were collected from AICRP on MULLaRP, Department of Genetics and Plant Breeding, IGKV, Raipur and one variety was collected from farmer of randomly selected village. These varieties were DPL-62, IPL-81, JL-3, K-75, L-4076 and local variety.

Data presented in table 1 showed that the healthy seeds were highest in variety JL-3 (94.93%) followed by K-75 (94.17%), DPL-62 (93.77%), L-4076 (93.66%) and IPL-81 (85.01%) and minimum in local variety (85.01%). Maximum percentage of damaged or broken seeds were recorded in seed lot of local variety (6.02%) followed by L-4076 variety (0.27%) and minimum in JL-3 variety (0.08%). Maximum percentage of shrunken seeds were detected in seed lot of K-75 (2.54%) followed by IPL-81 variety (1.95%) and minimum in JL-3 variety (0.89%). Small or under sized seeds were maximum found in seed lot of local variety (5.02%), followed by IPL-81 (3.46%) and minimum in L-4076 variety (1.39%). Percentage of inert matter were recorded maximum in L-4076 variety (1.34%) followed by DPL-62 variety (1.00%) and minimum in local variety (0.24%). Maximum percentage of other crop seeds were found in seed lot of DPL-62 variety (0.57%) followed by JL-3 variety (0.42%) and minimum in seed lot of IPL-81 variety (0.07%).

Hence, among all the lentil varieties, JL-3 variety showed the maximum purity as compare to other lentil varieties taken in the study and local variety were found as minimum pure among all the varieties. Many seed borne pathogens have the ability of causing distortions, discolouration, shrinking and disease of seeds which were visible to the naked eyes and this was the concern to the farmers.

Variation in purity in general and lentil in particular depends on cropping situation, processing and storage etc. Hoque et al. (2014) evaluated lentil seed (BARI Masur-1) in dry seed inspection and seeds were categorized into healthy seeds, discoloured seeds, spotted seeds, unfilled seeds and deformed seeds. Sharma (2001) and Kesharwani et al. (2018) also detected variation in purity on pea seeds supports the findings of present study. Purity of seed lots of legume crop vary considerably as recorded by Kumar et al. (2017) in pulses (pigeonpea, black gram, green gram and gram); Pareek and Varma (2015) in cluster bean; Haider and Ahmed (2014) and Pradhan (2017) in mungbean; Kaur (2010) in chick pea and Pradhan (2019) in Indian bean corroborating with the findings of present study.

2. Washing test

To know the spore load present on lentil seed lots, washing test was performed and data presented in the table 2 indicate that the maximum spore load was found from seed lot of local variety i.e. 20×10^2 which includes eight spores of Rhizopus stolonifer, three spores of Aspergillus flavus, two spores each of Aspergillus niger, Chaetomium globosum, Trichoderma viride and Cladosporium sp. and one spore of Alternaria alternata. This was followed by seed lot of IPL-81 (18×10^2), L-4076 (15×10^2), DPL-62 (14×10^2) and minimum spore load found in variety of JL-3 and K-75 (13×10^2).

Overall, predominant mycoflora with highest spore load were Rhizopus stolonifer (37) followed by Aspergillus flavus (14), Chaetomium globosum (12), Cladosporium sp. (8), Trichoderma viride (7), Aspergillus niger (6), Curvularia lunata (5), Penicillium sp. (2) and Alternaria alternata (2).

In this method, mycoflora recorded which adhered on the seed surface. Mycoflora recorded associated with seeds of lentil in the present study were also reported by Sharma (2001) and Kesharwani et al. (2018) as mycoflora present on the seeds of pea; Rathod et al. (2012) tested the seed borne mycoflora of different varieties of legume seeds; Trivedi and Rathi (2015) and Kumar (2016) recorded the variation in the frequency of adhered mycoflora on the surface of chickpea seeds; Pradhan (2014) in pigeonpea; Pradhan (2017) in mungbean; Kumari and Saxena (2017) detected seed associated mycoflora on horse gram seeds and Pradhan (2019) in Indian bean through washing out test support the findings of present study.

Table 1: Seed health evaluation of different varieties of lentil by dry seed examination

S.No.	Varieties	Healthy seed (%)	Small seed (%)	Discoloured seed (%)	Broken seed (%)	Shrunken seed (%)	Inert matter (%)	Other crop seed (%)
1	DPL-62	93.77	1.69	2.05	0.10	0.92	1.00	0.57
2	IPL-81	89.48	3.46	4.00	0.25	1.95	0.79	0.07
3	JL-3	94.93	2.54	0.39	0.08	0.89	0.75	0.42
4	K-75	94.17	2.21	0.14	0.14	2.54	0.75	0.10
5	L-4076	93.66	1.39	1.85	0.27	1.25	1.34	0.27
6	Local variety	85.01	5.02	1.87	6.02	1.71	0.24	0.20

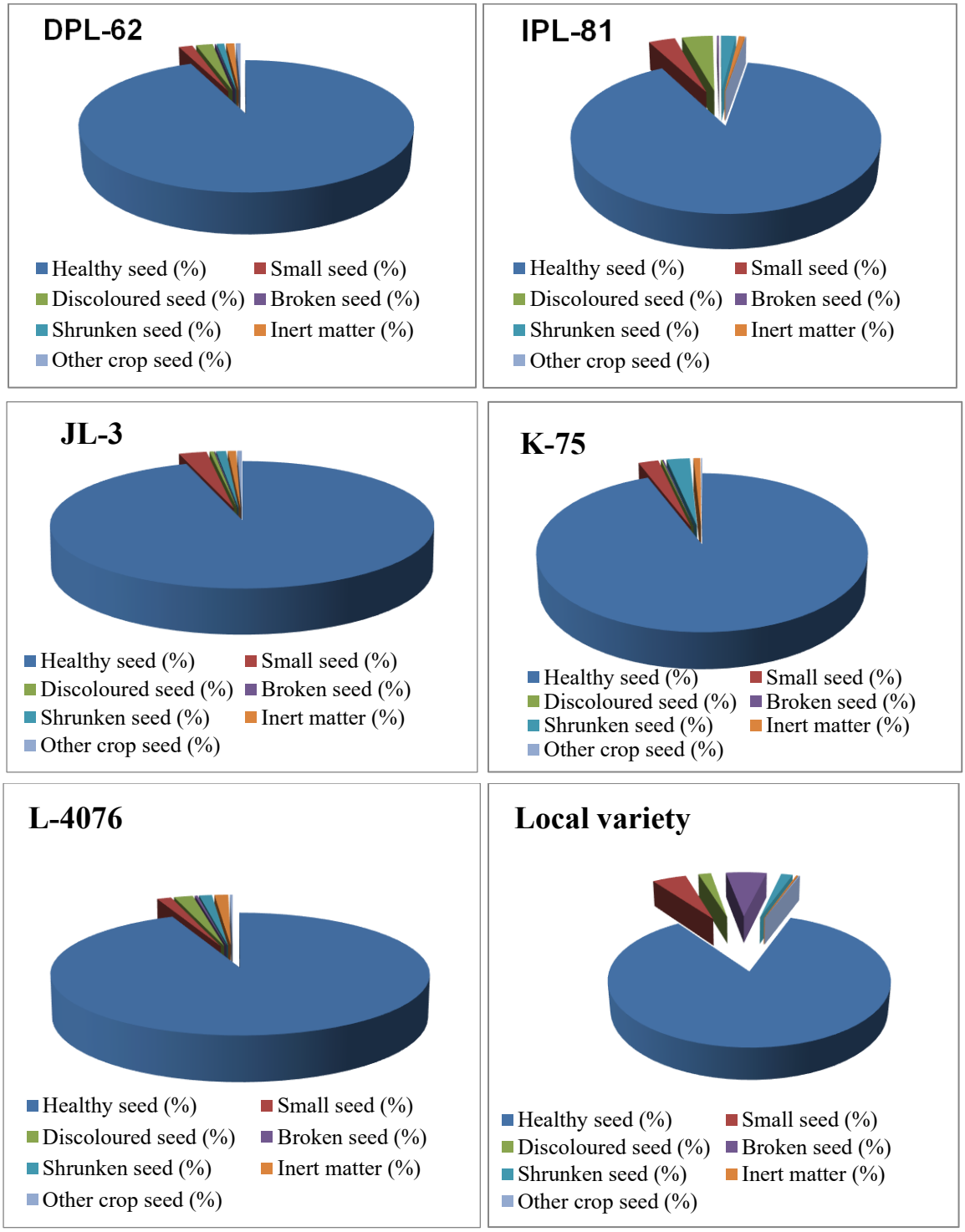
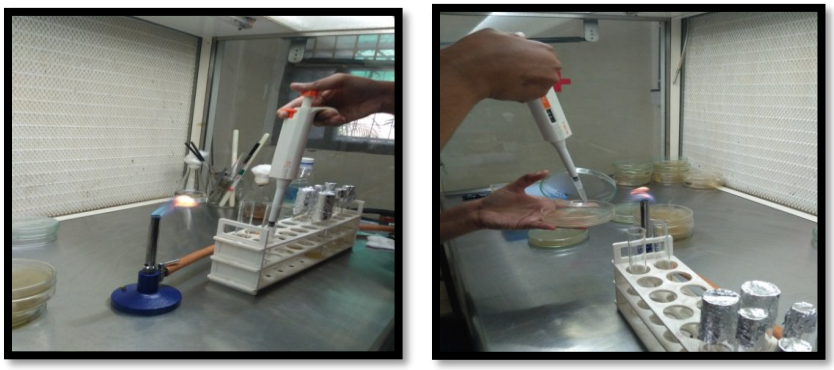


Figure 1: Seed health evaluation of lentil varieties by dry seed examination

(A) Dry seed examination



(B) Washing test

Figure 2: Procedure used during investigation for the assesment of seed borne mycoflora associated with lentil seeds

Table 2: Spore load of seed borne mycoflora on lentil seed lot (washing test)

S.No.	Varieties	Number of CFU (× 10²)									Total CFU (×10²)
		A. flavus	*A. niger*	*Chaetomium globosum*	*Rhizopus stolonifer*	*Alternaria alternata*	*T. viride*	*Penicillium* sp.	*Cladosporium* sp.	*C. lunata*	
1	DPL-62	2	1	2	6	-	1	-	1	1	14
2	IPL-81	2	2	1	7	-	2	-	2	2	18
3	JL-3	2	-	2	5	-	2	-	2	-	13
4	K-75	2	1	2	5	1	-	1	1	-	13
5	L-4076	3	-	3	6	-	-	1	-	2	15
6	Local variety	3	2	2	8	1	2	-	2	-	20
Total mycoflora		14	6	12	37	2	7	2	8	5	

IV. Conclusion

The conclusion from the results of study is as under

Seed health evaluation of varieties of lentil crop seed by using dry seed examination test and washing out test.

Seed lot of six different varieties of lentil showed variation in physical purity like, healthy seeds, damaged seeds, shrunken seeds, small or under sized seeds, discoloured seeds, inert matter and other crop variety seeds.

JL-3 variety (0.42%) and minimum in seed lot of IPL-81 variety (0.07%) in dry seed examination test.

In washing out test, seed lot of IPL-81 (18×10^2), L-4076 (15×10^2), DPL-62 (14×10^2) and minimum spore load found in variety of JL-3 and K-75 (13×10^2).

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