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ISOLATION OF SEED SURFACE FUNGI OF *LENS CULINARIS* (MEDIC)

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To isolate the seed surface mycoflora plate method of International Seed Testing Association (ISTA, 1966) and simple filter paper method were adopted. In which the seeds were taken and soaked for 2 hours in 0.02% aquous solution of 2,4-D for prevention of germination. These treated seeds were placed in the sterilized petriplate containing (A) Potato dextrose agar (PDA) medium and (B) sterilized wetted filter papers .These plates were incubated at 26±2°C temperature for one week. *Aspergillus* spp., and *Penicillium* spp., were most common and dominant fungi in all seed samples. *Alternaria* spp., *Rhizopus* spp., and *Fusarium* spp., were other frequent fungi present on seed sample.

Keywords: Filter paper; PDA; Plates; Seed.

The Lentil or Masur (Hindi) scientifically known as *Lens culinaris* (Medic) belong to family Fabaceae. It is recognized as a valuable pulse and is grown as a winter crop all over India. It is known to be the most nutritious of the pulses and an important item in the diet of some people, particularly those of eastern Bengal.

Lentil plant is erect, branched 25-40 cm tall leaves pinnately compound with 5-7 pairs of ovate leaflets, flower white pinkish or blue-purple, seed brown, grey or red colour with yellow or deep orange cotyledons. The flowers are borne in racemes of on short peduncles. The lower most buds open first and it takes nearly a fortnight, for the complete opening of all the flowers on a single branch. Flowers are papilionaceous and fruit is a pod.

Young pod is also eastern as a vegetable and the dry leaves and stalks are greatly prized as fodder. The seed have moisture 12.4%, protein 25.2%, fat 0.7%, carbohydrates 59.7% and mineral 2.1%. The carbohydrates present are hemi cellulose, starch, stachyose and reducing sugar.

The higher amount of protein in legumes is due to their atmospheric nitrogen fixation capability through the symbiotic *Rhizobium* bacteria found in their root nodules. Besides *Rhizobium* some other nitrogen fixing bacteria and *Actinomycetes* are also found in soil in free state along with many fungi and algae .The region in the vicinity of root can be distinguished into many algae in the rhizosphere, rhizoplane and non-rhizosphere zones. The Rhizoplane is the direct contractual area of the root after removing complete soil by frequent washings.

Seeds of Lentil were collected from three different major crop cultivating places of Rajasthan like Jaipur, Dholpur and Kauroli .All the seed samples were used for this investigation. Four hundred seeds of each sample were randomly drawn. These were examined by naked eyes as well as under stereo binocular microscope (10x-40x) for the presence of thin, shriveled seeds and discoloration such as brown black spots general blacking and microscleretia etc. The per centage incidence of each type of seed was recorded.

For this study two methods were employed namely

1. Standard blotter method - Four hundred seeds per sample, 200 untreated and 200pretreated with 1percent available chlorine from aqueous solution of sodium hypochlorite (NaOCl) for ten minutes were used. In preliminary experiment 1% and 2% available chlorine solution for 5 and 10 minutes with each concentration were tried. One percent available chlorine for ten minutes was found suitable and thus used throughout the experiment. Twenty five seeds were spaced in sterilized petriplate of 10 cm diameter containing three pieces of well moistened blotters. The plates were incubated at about 28±1°C under 12 hrs of alternating cycles of day light (from incubated Phillips fluorescent tube) and darkness for seven days. The seeds were examined under stereo binocular microscope on 8th day of incubation for seed-borne mycoflora and germination. In some cases incubation was prolonged and observation made on 12th and 15th days also.

2. Agar plate test - In all seed samples agar plate test method was employed. 200gm of peeled potato, 20gm dextrose and 20gm agar per liter of distilled water were

S. No.	Fungi	Percentage of seed surface fungi in non-sterilized condition				Percentage of fungi in seed after surface sterilization			
		Blotter paper		PDA		Blotter paper		PDA	
		Range	Average	Range	Average	Range	Average	Range	Average
1.	*Alternaria alternata*	11.2-20.9	16.59	10.4-23.3	15.9	9.8-15.8	15.8	14.9-19.9	16.8
2.	*Aspergillus candidus*	12.4-20.17	15.7	11.1-19.9	16.0	-	-	-	-
3.	*A.flavus*	16.6-22	19	17-22.9	19.0	-	-	-	-
4.	*A.fumigatus*	11-19	15.89	10.3-22	15	8-16	16	15-20	17.25
5.	*A.niger*	14-22	18	13-18	16.0	12.1-16.2	14.33	11.1-16	12.7
6.	*A.terrus*	10.2-11.5	10	10-13	10.99	-	-	-	-
7.	*Curvularia lunata*	9-10.66	9.5	11-12	11	-	-	-	-
8.	*Fusarium oxysporum*	8.2-13	11.5	10-11	10.32	7-17	10.1	8.5-11.6	10
9.	*F. udum*	7-14	11	8-14	10.55	5.5-19.5	10.55	8-13	11
10.	*Mucor* sp.	1.1-5.4	4.0	2.9-7.6	5.6	3.8-5.5	3.5	3.6-6.5	4.7
11.	*Penicillium notatum*	5.7-11.8	8.3	5-12.4	8.4	-	-	-	-
12.	*Rhizopus* sp.	6-12	9	6-11.4	7.99	-	-	-	-

used to prepare potato dextrose agar media (PDA).The mixture was autoclaved and 15to 20ml of autoclaved PDA was poured aseptically in to each oven sterilized Petri plate. Two hundred seeds per sample, treated with one per cent available chlorine aqueous solution of sodium hypochlorite for five minutes, were spaced aseptically, with twenty seeds per Petri plate containing PDA. Petri plates were incubated as under standard blotter method and for collection of data after 8 days.

Isolation of seed mycoflora :

A. Isolation of seed surface mycoflora - For isolation of seed mycoflora agar plate method of International Seed Testing Association (ISTA, 1966) was adopted. For isolation of seed surface fungi, seeds were taken and soaked for 4-5 minutes in 0.02% aqueous solution of 2, 4-D. This treatment was given to suppress the germination of seeds for few days. These 2, 4-D treated seeds were placed in sterilized petriplate containing potato dextrose agar medium or sterilized filter paper at the rate of twenty seeds per plate. These plates were placed at 28±1°C temperature and examined for the appearance of fungi after one week.

B. Isolation of Internal seed mycoflora - For isolation of internal fungi the 2, 4-D treated seeds were surface sterilized with 0.1% $HgCl_2$ solution for 3 minutes and washed several times with sterilized water. These seeds were dried on sterilized filter paper and plated on autoclaved potato dextrose agar medium or sterilized filter paper. These plates were placed at 28±1°C temperature and examined for the observation of fungi after one week.

C. Purification of fungi - Purification of fungi pour and streak plate method was used .sterilized media poured in petriplate and small quantity of fungi inoculums was streaked and rest eked on the plates. These plates were incubated at 28±1°C in B.O.D. for 6-8 days. The distinct colony that appeared away from the streak in second and third petriplate was sub cultured in slants.

D. Maintenances of pure culture - The fungi isolated were maintained on PDA slants and stored at 5°C in a refrigerator .these cultures were revived after consumption of media .the plugs were sealed with wax to avoid contamination and loss of moisture. Whenever required

the purification was done by streak plate method.

In present study the common genera such as *Aspergillus flavus* , *A.nigur* , *A.fumigatus, A.terrus* , *Aspergillus candidus, Curvularia sp., Fusarium oxysporum* , *Penicillium spp., Mucor sp., Rhizopus sp* ., *Fusarium udum* and *Alternaria alternata* were found to be the most dominant on the surface and in the seeds of lentil (Table 1).

Singh and Tripathy[1] studied the mycoflora associated with stored seeds of lentil using the blotter paper technique and the agar method on three different media (Czapeck's Dox Agar, Malt Agar and Potato Dextrose Agar) and isolated. *A.alternata, A.flavus, A.niger, C.globosum, Cladosporium herbarum, F.oxyporum* and *P.crysogenum.*

According to Christensen and Kaufmann[2,3] the fungi that invade the seed can be divided in to two general groups. Field fungi and storage fungi. Field fungi are the fungi which invade the seeds while they were developing on the plants in the field. Storage fungi are those that develop during prolonged storage without free water availablity. They comprise mainly of *Aspergillus* spp., and a few *Penicillum* spp.[4] and have considerable importance in the deterioration of storage grains[5].

Bansal and Sobti[6] discussed control of *Aspergillus flavus* associated with ground nut seeds. Antagonistic effect of micro organisms to *F.oxysporum* was studied by Dhedhi *et al.*[7]

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