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Prevalence of seed mycoflora from different varieties of Maize

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Seed-borne fungi of Maize in Kanpur region were surveyed. A total of 25 seed samples of eight different varieties from various locations, collected were tested, using the blotter and agar plate methods. Thirty two fungal species of twenty one genera appeared in the seeds of eight different variety of Maize. In untreated seeds of the entire varieties maximum incidence was of *Rhizoctonia solani*. Treated seeds showed complete absence of *Botrytis cinerea, A. nidulans*, *Alternaria alternata.* In Agar plate method was found to be favorable for the maximum counts of saprophytic fungi and also favorable for detection of some specific fungi. Presence of many pathogenic fungi in considerable number of seed samples indicates the need of field surveys for these and other pathogens.

Key words: Isolation, seed-borne fungi, maize, SDM, PDA

Seed samples of eight maize varieties namely Dabar 900, Kargil, Kaveri, Mukta, Pinucle, Rasi, Supper-900, and Vimal were collected from market places, field and storehouses from different parts of Kanpur nagar and dehat. A composite sample of each variety was prepared by mixing the individual samples together, preserved in cloth bags in laboratory conditions at room temperature during the studies.

The seed mycoflora was isolated by using Standard moist blotter method (SBM) and Agar plate methods (APM) as recommended by International Seed Testing Association (ISTA, 1996).

In Standard blotter method (SBM) pair of white blotter papers of 8.5cm diameter was jointly soaked in sterile distilled water and were placed in pre-sterilized petriplates of 10cm diameter. Ten seeds of test samples per petriplates were placed at equal distance on the moist blotters. One hundred seeds were tested for each treatment. The plates were incubated at 25±1°C under diurnal conditions for 7 days.

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Agar plate method (APM)

In pre-sterilized corning glass petriplates of 10 cm diameter were poured with 15 ml of autoclaved PDA medium. On cooling the medium, ten seeds per petriplates of the test sample were placed at equal distance aseptically. Incubation conditions and other details were same as described for the blotter method. In order to isolate only internal mycoflora, seeds were pretreated with 0.1% mercuric chloride for two minutes andsubsequently thoroughly washed thrice with sterile distilled water and placed on agar plates. Seeds without any such pretreatment were employed for the total seed mycoflora (control).

The fungi occurring on each and every seed in the plates were identified preliminary on the basis of sporulation characters like sexual or asexual spores with the help of stereoscopic binocular microscope. The identification and further confirmation of seedborne fungi was made by preparing slides of the fungal growth and observing them under compound microscope. The identification was made with the help of manuals. Pure cultures of these fungi were prepared and maintained on potato dextrose agar (PDA) slants.

Table 1 : Percent seed mycoflora of Maize (Blotter test method)

Fungal isolates	Maximum % incidence		Number of sample with which fungus associated (out of 8)
	Untreated	Pretreated	
Alternaria alternata	05	00	03
A. brassicicola	35	25	05
Aspergillus candidus	25	15	05
A. flavus	20	08	08
A. fumigatus	15	04	04
A. nidulans	05	00	05
Botrytis cinerea	08	00	08
Chaetomium brasiliense	03	08	02
Drechslera rostrata	15	32	03
Rhizoctonia solani	65	45	04
Sclerotium rolfsii	45	16	08
Trichoderma viride	20	32	05
Curvularia lunata	45	25	03
Epicoccum purpurascens	15	32	05
Nigrospora sphaerica	15	05	08
Phytopthora spp.	35	25	04
Pythium spp.	15	15	02
Rhizopus nigricans	45	15	08
Syncephalstrum spp.	15	02	05
Trichothecium roseum	15	00	03

Maize seed mycoflora (Blotter test method) A total of twenty fungal species belonging to fifteen genera found on eight different cultivars tested. In untreated seeds maximum incidence was of *Rhizoctonia solani* (65%) followed by *Rhizopus nigricans, Sclerotium rolfsii, Curvularia lunata, A. brassicicola, Phytopthora* spp., *Aspergillus flavus, Aspergillus candidus and Trichoderma viride* (15-45%) while *Aspergillus nidulans, Chaetomium brasiliense and Alternaria alternata* were reported poorly (Table I). Seed treated with surface sterilizer (0.1% $HgCl_2$) showed complete absence of certain fungi *(Alternaria alternata, Botrytis cinerea, Aspergillus nidulans, Trichothecium roseum*) or low incidence of *A. fumigatus, A. flavus, A. brassicicola, A. alternata, Drechslera rostrata, Sclerotium rolfsii*, etc. On the other hand counts of *Drechslera rostrata, Trichoderma viride, Epicoccum purpurascens and Chaetomium brasiliense* were found to be increased (Table 1). Similar types of observations have been made by Kushwaha (2014).

Table 2 : Percent seed mycoflora of Maize (Agar plate method)

Fungal isolates	Maximun % incidence		Number of sample with which fungus associated (out of 8)
	Untreated	Pretreated	
Alternaria alternata	07	00	04
A. brassicicola	05	07	05
Aspergillus candidus	15	09	08
A. flavus	45	12	05
A. fumigatus	25	05	03
A. terreus	15	02	04
Botrytis cinerea	35	00	08
Cephalosporium acremonium	25	00	04
Curvularia pallescens	04	05	05
Chaetomium brasiliense	15	09	06
Drechslera rostrata	45	32	05
Drechslera sorghina	15	14	05
Fusarium oxysporum	25	16	04
Gonatobotrys ramosa	20	16	08
Harmodendron spp.	08	03	08
Myrothecium roridum	16	22	06
Penicillium chrysogenum	15	16	04
Penicillium oxalicum	15	07	08
Rhizoctonia solani	20	08	08
Sclerotium rolfsii	06	00	05
Trichoderma viride	02	03	02
Curvularia lunata	03	00	06
Nigrospora sphaerica	04	00	05
Phytopthora spp.	05	08	03
Pythium spp.	04	08	04
Rhizopus nigricans	45	07	08
Trichothecium roseum	15	00	03
Torula herbarum	10	00	08
sterile mycelium	06	02	04

The data summarized in Table 2 shows that the

saprophytic mycroflora increased on agar medium, similarly some new fungi were detected which were absent in blotter test. These fungi are *Aspergillus terreus, Cephalosporium acremonium, Curvularia pallescens,Drechslera sorghina, Fusarium oxysporium, Gonatobotrys ramose, Harmodendron* spp., *Myrothecium roridum, Nigrospora sphaerica, Penicillium chrysogenum, P. oxalicum, Torula herbarum* and *Trichoderma spp*. On other hand *A. nidulans, Syncephalstrum spp.* and *Trichothecium roseum, Epicoccum purpura scens,* were not detected in agar plate method. The dominant fungi in agar test were *Aspergillus flavus, Botrytis cinerea, Drechslera rostrata, Cephalosporium acremo nium, Rhizopus nigricans, Fusarium oxysporum* (25-45%). The percent incidence of saprophytic mycoflora in agar plate method (Table 2) increased regularly, and also appeared some new fungi. This suggests that the above mycoflora might have appeared due to nutrients in the medium. Appearance of some new fungi, only on agar and which did not found in blotter method indicates that these fungi need some external supply of nutrients. In agar method *Aspergillus niger, Penicillium* spp*., Rhizopus arrhizus* suppressed the growth of other fungi of maize seeds. In most of cases agar plate was found to be superior than blotter for the isolation of seed mycoflora.

Agar plate method was found to be favorable for the maximum counts of saprophytic fungi and also favorable for detection of some specific fungi. The presence of so many pathogenic fungi at high levels in various geographical areas indicates a clear need for field surveys for these and other pathogens. There also is a clear need to increase public awareness on aspects related to seed health and to develop suitable management practices for improving the quality of the seeds. Testing seed health of major crops should be introduced in the national seed quality control system.

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