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Detection and transmission of seed borne fungi of maize

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Abstract
An experiment was undertaken to identify the seed borne fungi in maize and subsequently determine their effect on seed germination in laboratory condition. Seed samples were examined in blotter method showed association of twelve fungi belonging to ten genera viz., *Acladium* sp., *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodia theobromae*, *Curvularia lunata*, *Curvularia oryzae*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Penicillium* sp., *Rhizoctonia bataticola*, *Rhizopus* sp. and *Trichoderma viride*. In component plating, maximum Colonization of *Curvularia lunata*, *Fusarium moniliforme*, *Fusarium oxysporum* and *Rhizoctonia bataticola* predominantly noticed in seed coat and endosperm tissue of maize seeds. Under seedlings symptom test *Curvularia lunata*, *Botryodiplodia theobromae*, *Fusarium moniliforme*, *Fusarium oxysporum* and *Rhizoctonia bataticola* were found to transmissible from seed to plant causing seedling blight.

Keywords: Maize, detection, colonization, transmission

Introduction
Maize (*Zea mays* L.) belongs to the cereal family Graminaceae is one of the most leading cereal in the world and ranks third next to rice and wheat. Seed-borne pathogens are a serious threat to seedling establishment. Infected seed failed to germinate and may transmit pathogens to seedling and growing plant in field that interfere with production. As many as 112 diseases are known to occur in maize crops (USDA, 1960) where more than 70 diseases are seed-borne. Again of all the seed-borne pathogens, fungi are predominant and about 60 fungi are known to be seed-borne or seed transmitted in maize. The seed borne pathogens associated with seeds externally or internally and cause seed rot, seedling blight and resulting into low germination. Hence, in the present investigations the detection, location, transmission of seed borne fungi of maize was studied.

Material and Methods
Seed samples of maize were collected from farmers of different locations and APMC of Akola, Amravati, Aurangabad, Satara and Latur.

Detection of seed borne fungi: Blotter paper method
Standard blotter method (ISTA, 1985) [5] is widely used for the detection of seed borne fungi of most kinds of seed and hence the same method was employed. For this test, 400 seeds of each sample were sown on three layers of pre-soaked moist blotter paper having 9 cm diameter. In each plate 10 seed were arranged, 9 seeds in the outer ring and 1 in middle ring and one in the center of plastic plates. The plated seeds were then incubated at 27±2 ˚C, under alternate cycle of 12 hrs light and 12 hrs darkness for seven days by using two 40W white fluorescent tubes. After seven days of incubation, seeds were examined under stereoscopic microscope by using a magnification of 6X to 50X. Research microscope was also used to confirm the identification of fungi based on morphological characters given in standard mycological books.

Component plating method
This method was performed to know the site of infection of seed borne fungi. For this, seeds were selected randomly and washed four times with tap water. Then seeds were soaked in petri plates containing sterilized distilled water for 4 hrs. Individual seeds were aseptically dissected into three components viz. Seed coat, endosperm and embryonic region by using sterilized

blade every time. While dissecting seed coat, care was taken to avoid injury to endosperm so that there should not be possible infection from seed coat to endosperm. Thus, out of each sample 10 seeds were dissected. Components of each seeds were placed with sterilized forceps on solidified PDA medium in plates. Plated components were incubated under alternate cycle of light and darkness for seven days at room temperature and then were examined for presence of fungi by preparing slides under compound microscope.

Seedling symptom test: **Test tube agar method**

Symptoms can easily be studied being visible on seeds, roots as well as green parts. In each water agar stab, one seed was incubated at 27±2 ˚C under alternate cycle of 12hrs light and 12hrs darkness. To retain moisture, they were covered individually by aluminum foil which was removed when the seedlings has reached to cover. Thirty seeds of each variety were tested for detection of seed to plant transmission of seed borne fungi. The seedling was examined after 14 days for typical symptoms of disease on the various parts of seedling.

Isolation of seed borne fungi

Structure of seed borne fungi growing over incubated seeds were observed under stereoscopic microscope, lifted with sterilized needle and transferred aseptically on PDA media. Fungi were purified by hyphal tip method and maintained on PDA slants for further studies.

Result and Discussion

Seed samples of maize were recorded the association frequency of twelve seed borne fungi belonging to ten genera *viz.*, *Acladium* sp. (0-5%), *Aspergillus flavus* (6-14%), *Aspergillus niger* (5-14%), *Botryodiplodia theobromae* (0-14%), *Curvularia lunata* (1-6%) *Curvularia oryzae* (0-6.6%), *Fusarium moniliforme* (7-12%), *Fusarium oxysporum* (4-9%), *Penicillium* sp.(2-10.5%), *Rhizoctonia bataticola* (3.5-14%), *Rhizopus* sp. (3.5-14%) and *Trichoderma viride* (0-5%).

Seed sample of Satara (Dhebewadi) was recorded highest fungal association among all seed samples tested by blotter method. The total twelve fungi belonging to ten genera were recorded viz., *Aspergillus niger* (14%) followed by *Aspergillus flavus* (11%), *Fusarium moniliforme* and *Penicillium* sp. (10.5%), *Rhizopus* sp. (8%), *Fusarium oxysporum* (7.5%), *Rhizoctonia bataticola* (3.5%), *Acladium* sp. (3%), *Curvularia lunata* and *Trichoderma viride* (2.5%), *Curvularia oryzae* (2%) and *Botryodiplodia theobromae* and *Curvularia oryzae* (1%).

Seed borne fungal association in other samples of maize of different locations i.e. Samples of Akola, Aurangabad, Latur and Amravati were in range of 46-72%. Association of *Aspergillus flavus Aspergillus niger, Fusarium moniliforme, Fusarium oxysporum, Rhizopus* sp., *Rhizoctonia bataticola, Curvularia lunata, Penicillium* sp., *Trichoderma viride* and *Acladium* sp. were most frequent. Among seed borne fungi of maize, *Aspergillus flavus* (9.4%) *Rhizoctonia bataticola* (9.25%), *Fusarium moniliforme* (8.9%) observed as predominant fungi followed by *Aspergillus niger* (8%) and *Fusarium oxysporum* (6.15%). The association of seed borne fungi of present investigation are in agreement with Akonda *et al.* (2016) who recorded the association of nine fungi belonging to seven genera *viz.*, *Aspergillus flavus* (27.42%), *Aspergillus Niger* (23.47%), *Fusarium oxysporum* (20.55%), *Penicllium oxalicum* (18.37%), *Rhizopus stolonifer* (17.83%), *Fusarium moniliforme* (17.39%), *Curvularia lunata* (7.87%), *Bipolaris maydis* (3.50%) and *Alternaria alteranata* (2.74%). *Aspergillus* sp. and *Fusarium* sp. observed as pre dominant seed borne fungi of maize in present studies are supported by the finding of Rahmatzai and Saifulla (2011) [8] and Shirurkar and Wahegaonkar (2013) [9].

Maximum colonization of seed borne fungi was recorded in seed coat (1.00 to 6.00%) followed endosperm (1.04 to 7.00%) and embryonic region (1.00 to 2.25%). Among all seed samples *Curvularia lunata, Fusarium moniliforme, F. oxysporum* and *Rhizoctonia bataticola* (0.13 to 7.00%), predominantly located in all parts of seed component i. e. seed coat, endosperm and embryonic region. Present findings are in confirmation with earlier workers, Kumar and Agarwal (1998) [7] detected *Botryodiplodia theobromae, Curvularia lunata* and *Fusarium moniliforme* in all components of seeds of maize. Dawar *et al.* (2007) [4] recorded *Curvularia lunata, Fusarium moniliforme* and *F. oxysporum* in all components of chickpea seeds.

From rotten seed and seedling, tissue isolation was done on PDA to know the responsible fungi associated with seed rot and seedling blight. Under isolation, *Aspergillus flavus, A. niger, Botryodiplodia theobromae, Curvularia lunata, Fusarium moniliforme, F. oxysporum* and *Rhizoctonia bataticola* were obtained from rotten seed, whereas blighted seedling yielded *C. lunata, R. bataticola, F. moniliforme, F. oxysporum* and *B. theobromae*. Thus, studies indicated that among all seed borne fungi *C. lunata, B. theobromae, R. bataticola, F. moniliforme* and *F. oxysporum* were found responsible for seedling blight. Hence, it was proved that *C. lunata, B. theobromae, R. bataticola, F. moniliforme* and *F. oxysporum* were transmissible from seed to plant. Present findings are in confirmation with the earlier workers, Kumar and Agarwal (1997) [6] who reported transmission of *Botryodiplodia theobromae* from seed to seedling in maize. Basak and Lee (2002) [3] reported that *Fusarium moniliforme* and *Fusarium* sp. produced distinct seed rot and seedling infection symptom in maize crop.

Table 1: Association frequency of seed borne fungi with Maize seeds (Blotter paper method)

Seed borne fungi	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Avg. seed mycoflora
Acladium sp.	3.0	4.0	2.0	1.0	5.0	3.0	1.0	0.0	0.0	0.0	1.9
Aspergillus flavus	11.0	14.0	13.0	8.0	7.0	8.0	6.0	9.0	8.0	10.0	9.4
Aspergillus niger	14.0	11.0	9.0	8.0	9.0	6.0	7.0	5.0	6.0	7.0	8.2
Botryodiplodia theobromae	1.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	12.0	14.0	2.7
Curvularia lunata	2.5	3.5	4.5	2.0	3.0	2.0	3.5	1.0	6.0	5.0	3.3
Curvularia oryzae	2.0	2.0	1.0	6.6	2.0	4.0	3.0	3.5	0.0	0.0	2.41
Fusarium moniliforme	10.5	7.0	8.5	9.0	10.0	7.0	8.0	12.0	7.5	9.5	8.9
Fusarium oxysporum	7.5	4.0	9.0	3.0	6	8.0	4.0	5.0	6.0	9.0	6.15
Penicillium sp.	10.5	5.0	3.5	6.0	6.5	2.5	7.0	3.5	2.5	2.0	4.9

Rhizoctonia bataticola	3.5	4.0	7.5	12.0	13.0	14.0	11.5	10.0	12.0	5.0	9.25
Rhizopus sp.	8.0	4.5	9.0	0.0	7.0	5.5	0.0	9.0	6.0	11.0	6.0
Trichoderma viride	2.5	1.0	1.5	3.0	1.0	0.0	0.0	4.0	2.0	5.0	2.0

Table 2: Association frequency of seed borne fungi with different parts of maize seeds (Component plating method)

Sample no.	Seed borne fungi associated	Per cent association of seed borne fungi		
		Seed coat	Endosperm	Embryonic region
Sample 1	*A. flavus*	1.25	-	-
	F. moniliforme	2.00	2.00	1.00
	Acladium sp.	1.65	-	-
Sample 2	*C. lunata*	3.30	1.65	-
	F. moniliforme	6.00	3.00	1.50
	R. bataticola	2.00	5.00	1.00
Sample 3	*C. lunata*	5.00	2.65	-
	F. moniliforme	3.00	4.50	2.00
	F. oxysporum	2.55	1.50	0.19
Sample 4	*F. moniliforme*	4.50	7.00	-
	R. bataticola	4.00	3.00	1.00
Sample 5	*F. moniliforme*	2.00	4.00	-
	F. oxysporum	3.45	1.04	0.13
Sample 6	*C. lunata*	4.25	2.20	-
	F. moniliforme	1.00	3.00	2.00
Sample 7	*F. moniliforme*	4.00	5.00	1.50
	R. bataticola	4.50	2.50	-
Sample 8	*C. lunata*	4.00	1.75	-
	F. moniliforme	6.00	3.00	1.60
	F. oxysporum	5.79	1.34	0.15
Sample 9	*F. moniliforme*	4.75	5.50	2.25
	R. bataticola	3.00	3.00	-
Sample 10	*B. theobromae*	2.00	3.00	1.00
	F. moniliforme	3.00	5.50	-

Table 3: Effect of seed borne fungi on seeds and seedling of maize tested by test tube agar method

Sample no.	No. of seed tested	Percent emergence (%)	Seed rot (%)	Seedling blight (%)	Fungi associated	
					Seed rot	Seedling blight
Sample 1	30	80.00	20.00	3.00	*A. flavus, C. lunata, R. bataticola*	*C. lunata*
Sample 2	30	73.00	27.00	11.00	*A. flavus, C. lunata, R. bataticola*	*C. lunata*
Sample 3	30	83.00	17.00	3.00	*C. lunata , R. bataticola*	*C. lunata, R. bataticola*
Sample 4	30	70.00	30.00	7.00	*C. lunata, R. bataticola*	*C. lunata*
Sample 5	30	63.00	37.00	5.00	*A. niger , C. lunata, R. bataticola*	*C. lunata, R. bataticola*
Sample 6	30	77.00	23.00	12.00	*C. lunata, F. oxysporum, R. bataticola*	*F. oxysporum*
Sample 7	30	73.00	27.00	9.00	*C. lunata, F. oxysporum , F. moniliforme*	*F. moniliforme*
Sample 8	30	67.00	33.00	11.00	*F. moniliforme, R. bataticola*	*F. moniliforme*
Sample 9	30	60.00	40.00	5.00	*F. moniliforme , F. oxysporum*	*F. moniliforme*
Sample 10	30	90.00	10.00	14.00	*B. theobromae, F. moniliforme*	*B. theobromae, F. moniliforme*

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