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Fungal Load on *Zea mays* Seeds and their Biocontrol

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Abstract

Seed borne fungi of maize (*Zea mays*) were isolated from fifteen varieties on blotter and different agar media. Total eleven, twelve and twenty fungi were isolated from fifteen varieties of maize on blotter paper, Czepek dox Agar (CZA) and Potato Dextrose Agar (PDA) respectively. Seed mycoflora of abnormal seeds was studied. Maximum incidence of fungi was observed on discoloured seeds followed by cracked and shrunken seeds. Attempts were also made to control seed mycoflora by using *Trichoderma viride*. *Trichoderma viride* showed maximum inhibition zone against *Alternaria alternata*, *Fusarium oxysporum*, *Helminthosporium tetramera*, *Penicillium notatum* and *Rhizoctonia solani*.

Key words: Seed mycoflora, Maize, Discolored seeds, *Trichoderma*

Introduction

Maize (*Zea mays* L.) is a cereal crop widely cultivated throughout the world and greater weight of maize is produced each and every year than any other grain. The United States produces almost half of the world harvest whereas, other countries which grow maize are as wide spread as China, Brazil, France, Indonesia, Japan, Korea, Taiwan, Mexico, Egypt, Malaysia, Colombia, South Africa and India. Worldwide production of maize approximately over 614.3 million metric tones in 2003 while, in the year 2004, total production was recorded to be 642.6 million metric tones. These countries account for around 80% of total world production. The maize is also commonly known as corn.

Major consuming Nations of corn are China and USA. There has been continuous increase in the consumption demand of corn mainly owning to increase in the demand from meat and starch sector. There is growing requirement of maize from poultry sector where it is being used as feed. Important Nations as the major exporter of corn are USA followed by Argentina, Brazil, China, South Africa and Ukraine. USA dominates the International trade of corn as an exporter.

The world now produces sufficient food to feed every one, yet 840 million people do not have enough food. Crop loss due to pest and diseases accounts for 400 m US $ and disease alone amounts to 26%. The production is likely to decrease by 1.5% per year by 2020, and India may need 30% more for consumption. Currently 18-20% of the total seed requirements in India are being met out by both private and public sector. The remaining seed requirements are being the farmer's own saved seeds.

India's Maize production is in between 10-14 million tones, with 80-90% of the production being in the Kharif season. In India during 1994-95 it occupied 6105.8 hector area with production of 9117.5 hector and yield was 1493 kg/hector. Major states that contribute for maize production are Karnataka, Andhra Pradesh, Bihar, Punjab, Utter Pradesh and Madhya Pradesh and Maharashtra. Around 6.5 million tones (roughly 50% of total consumption) go for feed use, primarily for poultry field. Another one million tones of corn is being used by the starch industries. Considering the nutritive values of corn and entire plant, it is very popularly used as a fodder in different state of India.

Seeds are subjected to policies and legislation because they represented major values. Food production and food security are largely based on seeds. Seeds are a gift of nature, of past generation and diverse culture. It is our inherent duty to protect them and to pass them on to future generation. During crop improvement program high yielding varieties/planting material were exchanged world over to mitigate the food requirement of every increasing population. Such varieties, at the same time, introduction certain pathogens along with the planting material, which spread across the boundaries or within the country, became established in the seed stock as serious seed-born pathogen responsible for disastrous epidemics and causing major menace in food production. It is imperative to focus on seed health which is detrimental to reduce the production cost and considerable yield losses to sustain the food security. Food security not only refers to higher production but also the access of the quality food to the commonest of the common at an affordable price. It would ultimately require cutting the production cost (Vishunavat, 2009.)

Healthy seeds are important for the production of healthy crop. About 90% of the world food crops are being produced by using seeds. These seeds are also responsible for disease transmission. This happens either in the field or in the post harvest storage condition. Due to the seed borne fungi, seed get deteriorated which may cause a great economic loss. In the presence of seed borne pathogens several types of abnormalities occur in the seeds. Such seeds are rejected by seed industries and for agricultural purposes. Considering the fact attempt has been made to study the maize seed mycoflora and their eco-friendly management.

Material and Methods

Collection of seed samples

Seed samples fifteen of maize varieties namely, African tall, Allrounder, Dabar 900, Kargil, Kaveri, Mukta, Pinucle, Rasi, Seed tech, Supper-900, Sweet corne and Vimal are cultivated. However, African tall, Kaveri, Supper-900, Rasi and Allrounder were collected from market places, field and storehouses from different parts of Marathwada region of Maharashtra state. For the collection of seed samples the method described by Neergaard (1973) has been adopted. 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.

Isolation of seed mycoflora

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); De Tempe (1970), Neergaard (1973) and Agarwal (1976).

a) 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 $25\pm2^{o}C$ under diurnal conditions for 7 days.

b) Agar plate method (APM)

In this method, pre-sterilized corning glass petriplates of 10cm diameter were poured with 15ml of autoclaved PDA and CZA

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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 pre-treated with 0.1% solution of mercuric chloride for two minutes and subsequently thoroughly washed thrice with sterile distilled water and placed on agar plates. Seeds without any such pre-treatment were employed for the total seed mycoflora (control).

C) Identification of seed-borne fungi

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 seed-borne 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.

Study of antagonistic antagonistic potential of *Trichoderma viride* against seed borne fungi

Antagonistic potential of *Trichoderma viride* against test fungi was studied by dual culture method. An agar disc 5mm containing mycelium of *Trichoderma viride* was inoculated at the centre of PDA poured petriplates and culture discs of the test fungi were placed at the centre of the plate. Petriplates were incubated for a week at 25$\pm$ 1^{o}C plates without antagonists served as control. Two replicates were kept for each treatment and observation on colony diameter (mm) and formation of inhibition zone were recorded.

Results and Discussion

Table 1 Shows that, total eleven fungi were isolated from fifteen varieties of maize by using blotter paper method. Among these eleven fungi, four species were of *Aspergillus* genera viz. *Aspergillus flavus, A. fumigatus, A. niger, A. ustus, Aspergillus flavus* and *A. niger,* showed their quantitative dominance. Two species are of *Fusarium* genera viz*. Fusarium moniliformae* and *F .oxysporum*. Among which *Fusarium oxysporum* showed its quantitative dominance. *Helminthosporium tetramerae, Mucor globsus, Penicillium* sp., *Rhizopus stolonifer* were also found to be associated with some varieties of maize. Varieties Local-II, African tall, Rasi and Local I were found to be more susceptible to the incidence of fungi, followed by Mukta and 457, whereas, Supper 900 and Vimal showed minimum association of fungi which indicated that these two varieties might be disease resistance.

Twelve fungi were isolated on fifteen varieties of maize by using Czepek dox Agar medium. Among these twelve fungi *Alternaria alternata* showed higher incidence. Three species were of *Aspergillus* genera viz. *Aspergillus flavus, A. fumigatus, A. niger* showed their quantitative dominance. Two species were of *Fusarium* genera viz. *Fusarium moniliforme* and *F. oxysporum*, among which *F. oxysporum* showed higher incidence. Three species were of *Penicillium* genera viz. *Penicillium notatum, P. oxalicum* and *Penicillium purpurogenum*. Among these *Penicillium notatum* showed higher incidence. Two species were of *Rhizopus* genera viz, *Rhizopus stolonifer* and *R. nigricans* of which *Rhizopus nigricans* showed maximum incidence. *Mucor globusum* was found to be associated with some varieties of maize. Varieties African tall, Local I, Local II Rasi, Sweet corn, All rounder, Mukta were found to be more susceptible to the incidence of fungi followed by Seed tech, 457 A pinacle where as Kargill, Kaweri and Vimal showed minimum incidence of fungi (Table 2).

It is observed from table 3 that, twenty species of fungi were isolated from fifteen varieties of maize on PDA media. Among these two species were of *Alternaria* genera viz. *Alternaria alternata, A. tenuissma* of which *Alternaria alternata* showed maximum incidence, five species were of *Aspergillus* genera viz. *Aspergillus flavus, A. fumigatus, A. niger, A. terrus* and *A. ustus.* Among which *Aspergillus flavus* and *A. niger* showed higher incidence followed by *Aspergillus fumigatus, A. terrus* and *A. ustus*. Three species of *Penicillium* genera viz. *Penicillium notatum, Penicillium oxalicum and Penicillium citritum* were isolated. *Penicillium notatum* showed maximum incidence. Two species are of *Fusarium* genera viz. *Fusarium moniliforme* and *F. oxysporum*. Among these *Fusarium oxysporum* showed higher incidence, two species were of *Rhizopus* genera viz. *Rhizopus nigricans* and *R. stolonifer,* among these *Rhizopus nigricans* showed maximum incidence. *Cladosporium spp, Curvularia lunata, Helminthosporium tetramere*, *Mucor globosus*, *Rhizoctonia solani* and *Trichoderma viride* were found to be associated with some varieties of maize. Varieties Local I, II, African tall, Mukta, Rasi and Sweet corn were found to be more susceptible to the incidence of fungi followed by All Rounder, Dabar, Kaweri, Kargill, Seed Tech and Vimal.

Maximum incidence of fungi was found on discoloured seeds followed by cracked and shrunken seeds. Bold seeds showed fewer incidences of fungi, except kargill variety. Out of total thirteen species of fungi, *Aspergillus* with four species *Fusarium* with two species were dominated on discoloured seeds, followed by *Curvularia lunata, Helminthosporium tetramere, Penicillium purpurogenum*, *Rhizopus nigricans, Rhizoctonia solani, Trichoderma viride* and *Alternaria alternate* showed minimum incidence. However shriveled seeds shows moderate incidence of fungi (Table 4).

It is observed fro table 5 that, *Trichoderma viride* showed maximum growth inhibition of *Alternaria alternata, Fusarium oxysporum, Helminthosporium tetramera, Penicillium notatum* and *Rhizoctonia solani*. However in case of *Aspergillus flavus, A. niger, A. terreus* and *Curvularia lunata,* there were less inhibition occurred in the presence of *Trichoderma viride.*

Bujari and Ershad (1993) recorded the maize seed mycoflora of eleven samples, collected from seed producers, 183 isolates obtained, which included 23 species from 13 genera. The fungi isolated were *Aspergillus flavus, A. candidus, A. clavntus, A. niger, A. terreus Cephalosporium eltum, Melanosperzama, Mucar, Nigrospora oxyzae, Penicillium chrysogenum. P. citrinum, P. oxalicum, P. purpupogenum, Rhizopus oxyzae, Trichoderma viride, Ustilago maydis and U. zeae.* The predominant fungi were *Aspergillus*, *Fusarium* and *Penicillium* genera. Kumar and Agarwal (1998) were reported fourteen fungi associated with discoloured seeds of different maize varieties, viz *Alternaria alternata, Aspergillus flavus, A. niger, Bipolaris carbonum, Bipolaris maydis, Botryodiplodia theobromae, Curvularia lunata, C pallescens, Epicoccum nigrum, Fusarium moniliforme, F. pallidoroseus, Rhizoctonia solani, Sclerotium rolofsi, Trichoderma harzianum*.
Somda *et al*. (2008) were detected ten pathogenic fungi from naturally infected maize seed samples, viz. *Acremonium strictum, Bipolaris maydis, Botryodiplodia theobromae, Colletotrichum graminicola, Curvularia spp.*, *Exserohilum rostratum, Fusarium moniliforme, F. equiseti, F. pallidoroseum, Phoma spp*, *Penicillium sp* and *Rhizopus sp*. which were common in the field.

Kulwant Singh, *et al*. (1987) was detected *Drechslera maydis* infected maize kernels from tribal areas of Rajasthan. Nishant Asif and Mall (2008) showed the presence of fifteen species belonging to eleven genera. Highest percentage of *Aspergillus niger* was recorded by him. Rao, *et al.* (2008) was detected ten species of *Fusarium.* Incidence of *Fusarium* was comparatively more in pre-harvest samples than in post-harvest samples. Species of *Fusarium* were *F. acuminutum, F. equiseti, F. moniliforme, F. graminearum, F. oxysporum, F. heterosporum* and same other fungi *Aspergillus flavus, A. fumigatus, A niger, A. candidus, A japonicus, Cladosporium, Rhizopus, Curvularia, Alternaria, Helminthosporium, Penicillium* were also observed.

Discolouration of the seed pericarp may be either due to physical presence of the pathogen or due to biochemical actions of the microorganisms. Blackening of jowar grains in the field due to dense infection of C*urvularia lunata* on the seed surface. Seed mycoflora, in general at initial stages appeared as white or grey mycelial growth on rachis, *glumes* and anthesis, while during sporulation various types of seed discolouration as blackening (*Curvularia*), Pinkish (*Fusarium*), show white (*Olpitrichum*), grey (*Alternaria* and *Drechslera*) are caused.

Fungi like *Phoma and Colletotrichum* produce small raised black dots over the pericarp which cause unsightly appearance of the grain (Rao and Williams 1978, Castor and Frederiksen 1981). Blackening of bajra grains in the field was always due to growth of *Curvularia lunata* and *C. penniseti* on the seed surface (Mathur *et al.,* 1960) while it was also due to other fungi like *Curvularia pallescens* (Bhatnagar, 1971), *Alternaria alternata, Drechslera tetramera, Penicillium* spp. and *Rhizopus nigricans* (Randhawa and Aulakh, 1984).Panchal (1984) stated in case of jowar that seeds with mixed type of discolourations showed higher counts of *Alternaria, Curvularia* and *Peniciliium* in agar plate. Chavan and Danai (1993) were isolate 15 fungal sp, from discoloured oil seeds *Alternaria tenuis, Aspergillus flavus, A. niger, A. fumigatus, Fusarium moniliforme* and *Rhizopus nigricans* were reported to be in predominant.Seed rotting is attributed mainly to the microbial destruction particularly with the help of their hydrolytic enzymes. This has been reported in various crop like cereals (Grewal and Mahedrapal., 1965, Mishra and Mishra., 1971), pulses (Sawhney and Aulakh, 1980, Bhikane and Mukadam, 1982), and oil seeds (Shukla and Bhargava 1977). The active group of fungi for seed rotting in different crops are as *Helminthosporium retrasiae, Curvularia lunata* and *Rhizoctonia bataticola* (Grewal, and Mahndrapal, 1965), *Aspergillus flavus, A. niger, Fusarium moniliforme* and *Penicillium* spp, for maize seeds (Aulakh *et al.* 1976), *Drechslera oryzae* for rice (Hiremath and Hegde, 1981) *Phoma insidosa* and several species of *Fusarium* (Suryanarayana 1978), *Helminthoporium hawiiensis* (Mishra and Mishra 1971), *Curvularia lunata* (Bhale and Khare, 1982), *Colletotrichum graminicola* (Basuchoudhary and Mathur 1979) for jowar have been recorded. Rati and Ramlingam (1974) found that *Aspergillus flavus* caused severe seed rotting irrespective of the 28 tested crops. Seed rotting in bajra has been reported due to *Alternaria alternata, Curvularia lunata, C. pallescens, Penicillium spp. Drechslera hawiiensis, D. longiroshtrats, D. maydis* and *Phoma*. Among the fungi the species of *Trichoderma* are the most important biocontrol agents, because they control various root disease caused by a wide rage of fungal pathogens (Alagarsamy *et al.,* 1987), (Mathivanan *et al.,* 2005), Similarly, Weindling, (1932) reported for the first time, the potential of *Trichoderma* as an effective biocontrol agent against soil borne fungal pathogens. Later several researchers across the world have demonstrated the control of a wide rage of plant pathogen using different species of *Trichoderma*. Similarly, Mukhopadhyay and Chandra (1985) firstly reported the biocontrol methods for control of tobacco damping off by *Trichoderma harzianum*. Raguchander *et. al.* (1993), showed that dry root rot in mung bean caused by *Macrophomina phaseolina* was control by the applications of biocontrol agent *Trichoderma viride*. Pushapavati and Chandrasekharrao (1999) tested the *Trichoderma spp.* i.e. *T. viride, T. harzianum* against *Sclerotium rolfsii* the incidence of groundnut stem rot. Kore and Chavan (2000) observed the efficacy of *Trichoderma* species in the management of safflower charcoal rot disease. *T. hamatum* was found more effective and inhibits the growth of *Macrophomina phaseolina,* D'couza *et al.,* (2001) screened *T. harzianum* against major fungal pathogens of betal vine ice *Phytophthora parasitica, Colletotrichum capsici, Sclerotium rolfsii* and *Rhizoctonia solani*. Where as, Gupta *et al*. (2002) studied the antagonistic properties of *Penicillium* sp, against different fungi viz, *Fusarium, Curvularia, Pestalotiopsis, Aspergillus, Hemilica* and a gram positive bacterium. Recently, Swami and Mukadam (2004) observed the efficacy of *T. viride* against the tomato fungi (*Alternaria solani, Geotrichum candidum, Phytophthora* sp., *Fusarium oxysporum, Aspergillus niger* and *Rhizopus stolonifer*). Similarly, Patale (2005) showed the antagonistic potency of *T. viride, T. harzianum* and *T. hamatum* against *Aspergillus niger, A. flavus, Rhizoctonia* sp. *Rhizopus* sp, and *Mucar* sp, Ukey *et al.,* (2004) suggested that by seed treatment and foliar sprays of *T. viride*, major disease of cotton such as root rot (*Rhizoctonia solani*), wilt (*F. oxysporum, Fusarium* sp. vasinfectum) bacterial blight (*Xanthomona axonopodis* pv. Malvaceanum), leaf spots (*Myrothecium rordum*) and *Alternaria macrospore* were significantly controlled eco friendly. Jhumadas and Ramarao (1990) showed in order to reduce the percentage incidence of seed borne fungi, maize seeds were inoculated with spore suspension of known antagonistic fungi viz. *Chaetomium globosum* and *Trichoderma viride* separately treated seeds reduced the number of seed borne fungi from 26 (Control) to only 4.Gajbe and Lanjewar (1989) studied antagonistic behaviour of *Aspergillus niger* in seed borne fungi associated with two rice cultivars and result showed *A. niger* had an over all inhibitory effect on the growth of many of the isolates. The clear inhibitory behaviour forming zone of inhibition of *A. niger* against *M. phaseolina, P. glomerata, P. sorghiae, C. lunata, D. oryzae, F. moniliforme* and *A. alternata*.

Table 1 Incidence of fungi of maize varieties on Blotter Paper

Fungi	Maize Varieties														
	African tall	**All rounder**	**Dabar 900**	**Kargill**	**Kaweri**	**Local I**	**Local II**	**Mukta**	**Pinucle**	**Rasi**	**Seed tec**	**Super 900**	**Sweet corn**	**Vimal**	**Var. 457**
Alternaria alternata	9	-	-	-	10	6	6	-	-	6	-	-	-	-	10
Aspergillus flavus	46	40	40	40	40	30	47	38	50	28	30	40	40	45	60
Aspergillus fumigatus	22	15	-	-	-	21	8	-	-	6	-	-	10	-	-
Aspergillus niger	28	55	30	10	-	3	36	33	40	10	30	10	40	35	20
Aspergillus ustus	8	-	-	-	-	8	5	10	-	7	-	-	-	-	10
Fusarium moniliforme	15	-	-	-	-	13	24	15	-	15	40	-	30	-	20
Fusarium oxysporum	10	10	-	45	25	10	20	16	10	16	35	-	35	-	25
Helminthosporium tetramere	5	-	-	-	-	-	12	11	-	5	-	-	-	-	-
Mucor globosus	14	-	-	-	-	8	9	13	-	13	-	-	-	-	-
Penicillium sp.	5	-	-	-	-	5	4	-	-	-	-	-	-	-	-
Rhizopus stolonifer	26	10	10	-	-	15	16	20	10	18	22	-	-	10	-

Table 2 Incidence of fungi of maize varieties on CZA medium

Fungi	Maize Varieties														
	African tall	**All rounder**	**Dabar 900**	**Kargill**	**Kaweri**	**Local I**	**Local II**	**Mukta**	**Pinucle**	**Rasi**	**Seed tec**	**Super 900**	**Sweet corn**	**Vimal**	**457**
Alternaria alternata	12	10	10	10	15	10	11	13	20	10	10	15	20	15	22
Aspergillus flavus	48	45	45	30	60	48	51	38	55	30	41	60	30	55	60
Aspergillus fumigatus	20	20	10	-	-	20	13	-	-	8	10	10	20	-	10
Aspergillus niger	21	55	45	10	-	32	34	39	50	17	22	40	30	50	20
Fusarium moniliforme	34	10	-	15	-	35	22	28	10	30	7	-	38	10	35
Fusarium oxysporum	11	10	-	40	36	13	21	24	-	15	48	20	50	20	32
Mucor globosus	8	-	-	-	-	8	11	11	-	12	-	-	-	-	-
Penicillium notatum	8	10	10	-	10	10	8	10	-	12	10	10	10	-	10
Penicillium oxalicum	10	10	-	-	-	8	8	10	-	10	-	-	10	-	10
Penicillium purpupogenum	9	-	-	-	-	8	8	-	10	12	-	-	9	10	-
Rhizopus stolonifer	25	25	-	-	-	22	17	24	20	23	20	10	25	-	25
Rhizopus nigricans	10	10	-	-	-	10	-	10	10	-	-	-	10	-	-

Table 3 Incidence of fungi of maize varieties (PDA medium)

Fungi	Maize Varieties														
	African tall	**All rounder**	**Dabar 900**	**Kargill**	**Kaweri**	**Local I**	**Local II**	**Mukta**	**Pinucle**	**Rasi**	**Seed tec**	**Super 900**	**Sweet corn**	**Vimal**	**457**
Alternaria alternata	13	15	10	10	20	12	10	11	20	7	30	15	22	15	12
Alternaria tenuissima	10	-	-	-	10	10	-	-	10	-	-	-	10	-	-
Aspergillus flavus	47	40	50	30	80	49	52	42	45	35	42	60	20	50	85
Aspergillus fumigatus	18	25	-	10	10	23	21	-	10	12	15	10	20	10	10
Aspergillus niger	26	60	55	10	10	50	30	33	55	15	30	40	10	50	19
Aspergillus terrus	10	-	10	10	15	20	15	10	-	-	10	10	-	10	-
Aspergillus ustus	9	-	-	10	-	10	-	21	-	13	10	-	11	10	10
Cladosporium	-	-	-	10	-	-	10	-	-	-	-	-	-	-	-
Curvularia lunata	-	10	-	-	-	10	-	10	-	-	-	10	10	-	-
Fusarium moniliforme	40	10	10	10	-	30	27	30	15	22	70	-	32	-	30
Fusarium oxysponim	13	15	15	50	40	14	20	22	25	17	42	20	40	10	25
Mucor globosus	9	-	-	-	-	10	10	10	-	10	-	-	-	-	-
Penicillium notatum	9	10	-	-	20	12	9	10	25	10	20	10	10	20	12
Penicillium oxalicum	8	-	-	-	-	10	8	9	10	9	-	-	12	10	10
Penicillium purpugenum	6	-	-	-	10	9	9	8	-	10	-	-	10	-	-
Rhizocotina solani	10	-	-	10	10	-	10	-	-	-	10	-	-	-	-
Rhizopus nigricans	30	20	20	-	-	20	21	22	20	24	20	10	25	25	25
Rhizopus stolonifer	-	-	10	-	10	-	10	-	-	-	-	10	-	-	-
Trichoderma viride	10	-	-	-	-	10	-	10	-	-	-	-	-	-	-

Table 4 Incidence of seed borne fungi on abnormal maize seeds.

Fungi	**Bold Seeds**			**Cracked / Damaged Seeds**			**Shunken / Wrinked**			**Discoloured seeds**		
	African tall	**All rounder**	**Kargil**	**African tall**	**All rounder**	**Kargil**	**African tall**	**All rounder**	**Kargil**	**African tall**	**All rounder**	**Kargil**
Alternaria alternata	-	-	-	-	-	-	-	-	-	05	-	05
Aspergillus flavus	15	10	20	20	15	25	20	20	25	25	15	30
Aspergillus fumigatus	10	-	15	15	10	15	10	10	10	15	10	15
Aspergillus niger	15	15	25	25	20	35	30	20	30	30	25	40
Aspergillus terreus	-	-	10	10	-	10	10	-	10	10	-	10
Curvularia lunata	-	-	15	15	-	15	15	10	15	20	-	25
Fusarium moniliforme	10	-	10	10	-	10	10	-	10	-	-	10
Fusarium oxysporum	20	20	30	30	20	40	20	10	20	10	-	10
Helminthosporium tetramera	-	-	-	-	-	-	10	-	-	10	10	15
Penicillium purpurpogenum	10	-	10	10	-	10	-	-	10	15	05	10
Rhizopus nigricans	20	10	25	25	10	30	15	10	25	-	-	-
Rhizoctonia solani	-	-	10	10	-	-	-	10	20	-	-	20
Trichoderma viride	-	-	05	05	-	-	-	-	05	-	-	10

Fungi	Bold Seeds		Cracked/Damaged Seeds		Shunken/Wrinked		Discoloured Seeds	
	Rashi	Sweetcorn	Rashi	Sweetcorn	Rashi	Sweetcorn	Rashi	Sweetcorn
Alternaria alternata	-	-	-	-	-	5	-	10
Aspergillus flavus	10	20	15	25	-	10	10	25
Aspergillus fumigatus	-	10	-	10	-	10	10	15
Aspergillus niger	15	25	20	35	15	15	25	40
Aspergillus terreus	-	10	-	10	10	15	10	15
Curvularia lunata	10	-	10	10	-	-	15	15
Fusarium moniliforme	20	25	20	30	10	10	30	25
Fusarium oxysporum	15	20	20	20	10	15	25	30
Helminthosporium gram	-	-	10	-	5	-	15	10
Penicillium purpupogenum	10	15	15	15	-	10	20	20
Rhizopus nigricans	10	10	10	20	10	15	20	30
Rhizoctonia solani	-	10	-	10	-	-	20	25
Trichoderma viride	-	-	-	10	-	5	10	10

Table 5 Antagonistic nature of Trichoderma against fungi

Fungi	Zone of inhibition due to *Trichoderma viride* (mm)	Control growth without *Trichoderma viride* (mm)	Percent Inhibition
Alternaria alternata	50	75	66
Aspergillus flavus	20	85	23
Aspergillus niger	15	82	18
Aspergillus terreus	25	74	33
Curvularia lunata	40	86	46
Fusarium oxysporum	40	65	61
Helminthosporium tetramera	34	68	50
Penicillium notatum	41	75	54
Rhizoctonia solani	35	70	50

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