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SEED-ASSOCIATED MYCOFLORA OF SOYBEAN FROM THE MARATHWADA REGION

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ABSTRACT:

Traditional and improved Soybean cultivars collected from different parts of the Marathwada region in Maharashtra in India were raised for studying grain discoloration and screening of seed-borne mycoflora. Ten species of fungi were isolated from a discolored grain of the samples using the standard blotter paper method. The most predominant species observed was *Fusarium spp.* followed by *Aspergillus Niger* and *Alternaria alternata*. The seed mycoflora shows significant diversities in all the cultivars. High accumulations of mycoflora were observed in traditional than improved cultivars. The association of the mycoflora with various cultivars indicated seed contaminations and thus reducing seed quality.

KEYWORDS: Soybean seed, germination, grain discoloration, quality seeds, seed-borne mycoflora

INTRODUCTION:

Soybean (*Glycine max* L.) It is the most important source of plant proteins in the human diet. Tervet (1945) found *Alternaria* on soybean seeds, Singh *et al.,* (1973) isolated the species of *Alternaria* from abnormal soybean seeds. Similarly, Sundaresh and Hiremath (1978) isolated *Alternaria* from soybean seeds "Soybean seeds have a greater nutritional value as it is a major source of protein and vegetable oil. Rao T V, Rajeswari B, Keshavulu K, Varma VS (2015) revealed that it contains 40-42% proteins, 20-22% oil, 21% starch, vitamins- A, B, C, D & K besides essential amino acids like lysine (5%) and a small amount of calcium, phosphorous, magnesium and iron . The species of Aspergillus, *Penicillium, Fusarium, Rhizopus and Alternaria* have been found commonly occurring as post-harvest molds in storage condition (Mehrotra and Aggarwal, 2003). Most of the species of Aspergillus are dominant and play vital role in the seed biodeterioration (Chavan, 2011). Study of I.S.T.A. Seed health testing of Seed Science and Technology (2016) states the quality of seeds is affected by seed borne mycoflora. The attack of plant pathogens is one of the reasons for the low productivity of soybean. Most economically important plant pathogen is transported from one region to another through seeds or propagating materials. Seed-borne diseases are commonly occurring during storage periods if the seeds are stored in a moist dark place. Rahman S, Vearasilper S, Srichuwong S. detection of Seed-Borne Fungi in Mungbean and Blackgram Seeds found that the pathogens can be found in seeds after or before the germination of seeds. The seed-borne disease can be spread through wind, water, insect, agricultural equipment and transportation. Suresh Kumar Sahu at el (2023) Studies on Seed Borne Mycoflora of Soybean Seeds by Incubation Methods they found that different incubation methods employed for the detection of seed-borne fungal infections of soybean. Germination and seedling vigor are reduced by seed-borne mycoflora of soybean and they can destroy or affect

grains during storage and become not suitable for human consumption. Some seed-associated fungi can affect the seedling or plant resulting in a decrease in productivity capacity. The seeds of fungal flora play a significant role in determining seed quality and longevity. Among all mycoflora of soybean *Alternaria alternata, Alternaria tenuissima. Aspergillus niger, Aspergillus flavus, Rhizopus spp., Fusarium spp., and Curvularia lunata* reduces the germination and seedling vigor to a greater extent compared to others and they can spoil the quality of grain during storage. The aim of the study on seed-borne mycoflora of soybean seeds using incubation methods is to investigate and identify the fungal species present in soybean seeds. This type of study is conducted to assess the quality and safety of soybean seeds used for cultivation.

MATERIAL AND METHODS:

1) Collection of seed samples

For the collection of seed samples, the method described by Neergaard (1973) has been adopted. Accordingly, random samples of different varieties of seeds were collected from fields, storehouses, marketplaces, and seed companies. A composite sample of each variety was prepared by mixing the individual samples and preserved in cloth bags in laboratory conditions at room temperature during the studies.

2) Detection of seed mycoflora

The seed mycoflora was isolated by using the standard moist blotter method (SBM) and Agar plate methods (APM) as recommended by the International Seed Testing Association (ISTA 1966); De Tempe (1970), Neergaard (1973) and Agarwal (1976).

a) Standard blotter method (SBM)

A pair of white blotter papers of 8.5cm diameter was jointly soaked in sterile distilled water and were placed in pre-sterilized Petri plates of 10cm diameter. Ten seeds of test samples per Petri plate were placed at equal distances on the moist blotters. One hundred seeds were tested for each treatment. The plates were incubated at 25±2oC under diurnal conditions for 7 days.

b) Agar plate method (APM)

In this method, pre-sterilized corning glass Petri plates of 10cm diameter were poured with 15 ml of autoclaved potato dextrose agar (PDA) medium. On cooling the medium, ten seeds per Petri plate of the test sample were placed at equal distances aseptically. Incubation conditions and other details were the same as described for the blotter method.

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 seed in the plates were identified preliminary based on sporulation characteristics like sexual or asexual spores with the help of a 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 the 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.

RESULT AND DISCUSSION

In the present study ten fungi were associated with the soybean seed including *Alternaria alternata, Alternaria tenuissima. Aspergillus flavus Aspergillus niger, Curvularia lunata, Fusarium moniliforme, Fusarium oxyspermum, Mucor, Penicillium spp.* And *Rhizopus spp.*The frequency of occurrence is high. The maximum number of fungi were isolated from the variety of soybean JS-9752 (26.24%), followed by MAUS-162 (25.56%), and ES-BALTIMOR-II (21.14%)as shown in table no.1.The occurrence of mycoflora ranges from low to high due to time duration during the storage period. Storage of seed causes maximum deterioration.

Table No.1 Percentage of Seed discoloration due to seed mycoflora

Varieties of Soybean	% Gain Discoloration
MAUS-71	9.08
MAUS-162	25.56
MAUS-725	9.98
MAUS-731	18.04
ES-BALITIMOR-II	21.14
JS-9752	26.24
DS-228	18.64
AMS-1001	10.00
AMS-100-39	3.21
KDS-753	2.83

Table No.2 Fungi Isolates from discolored seeds of Soybean varieties.

Mycoflora / Varieties	*Alternaria alternata*	*Alternaria tenuissima*	*Aspergillus flavus*	*Aspergillus niger*	*Curvularia lunata*	*Fusarium moniliforme*	*Fusarium oxyspermum*	*Mucor*	*Penicillium spp.*	*Rhizopus spp*
MAUS-71	17.3	8.2	20.4	15.2	9.2	13.5	9.8	5.1	1.9	7.4
MAUS-162	16.3	7.5	24.6	18.6	8.3	10.5	12.1	7.1	1.0	6.4
MAUS-725	21.9	15.3	30.4	11.5	19.1	11.9	19.7	8.0	3.0	8.2
MAUS-731	23.1	10.2	33.7	26.1	14.1	17.4	21.1	10.0	2.1	8.2
ESBALITIMOR-II	14.9	8.1	25.7	21.3	10.8	12.0	13.1	8.6	1.3	7.0
JS-9752	13.6	17.1	22.9	14.3	11.8	9.8	11.6	9.6	1.9	7.7
DS-228	15.4	19.7	5.2	16.3	17.2	10.0	17.9	9.2	1.4	8.0
AMS-1001	9.3	18.5	4.2	12.4	3.4	4.5	0.5	0.1	0.5	6.1
AMS-100-39	11.4	5.1	21.2	13.9	5.9	6.5	10.7	5. 08	4.5	7.5
MEAN	15.91	13.90	20.92	16.62	11.08	10.67	12.94	7.21	1.95	7.38

Table n. 2 shows that in all the varieties of soybeans associated with the seed mycoflora only the level of infection is different in each variety. Aspergillus favus is highly infected with each variety of soybeans instead of Mucor has very low infection to all varieties.

Competing Interest
The author did not receive support from any organization for the submitted work. No funds, grants, or other support was received.

Ethics Approval and Consent to Participate –
This is an observational study, no ethical approval is required. Research does not involve any human subject.

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