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Detection of Seed Mycoflora of Soybean by Seed Health Testing Methods

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ABSTRACT

An investigation was conducted to detect the associated seed mycoflora in soybean and its control. A total of 5 varieties of soybean seed were collected from Oilseeds Research Station, Latur and Seed Processing Unit (National Seed Project) VNMKV, Parbhani. Standard agar plate methods and modified PDA method were used for detection of seed mycoflora of soybean seeds. Across the two methods adopted, a total of six fungal genera including *Fusarium verticillioides*, *Macrophomina phaseolina*, *Alternaria alternata*, *Aspergillusniger*, *Aspergillus flavus* and *Rhizopus stolonifer* with the seeds of soybean were detected. The fungi detected were identified based on their cultural and morphological characteristics. Among the six fungal species detected the occurrence of *Fusarium verticillioides* was observed (38.00, 26.00, 28.10, 29.50 and 15.00%, respectively) with MAUS-71, MAUS-158, MAUS-162, MAUS-612 and JS-335 followed by followed by the fungi *M. phaseolina* (32.00, 15.00, 19.75, 22.00 and 12.50 %, respectively) in Standard agar plate method. In modified PDA method maximum frequency of *F. verticillioides* was observed (27.50, 25.00, 30.00, 32.50 and 28.00%, respectively) with MAUS-71, MAUS-158, MAUS-162, MAUS-612 and JS-335 followed by followed by the fungi *A. flavus* (37.50, 12.50, 2.50, 7.50 and 35.75 %, respectively). Per cent infectivity of seed mycoflora varied across the methods adopted and varieties tested. The highest per cent infectivity of 38.00% was observed with the fungus *F. verticillioides* on MAUS-71 in Standard agar plate methods. In modified PDA method the highest per cent infectivity of 35.75% was observed with the fungus *A. flavus* on JS-335.

Key words: Soybean, Seed Mycoflora, Standard agar plate method, Modified PDA method, *Fusarium verticillioides*, *Macrophomina phaseolina.*

Introduction:

Soybean (*Glycine max*. (L) Merril) a grain legume is widely grown crop due to its high quality protein and edible oil. Soybean is presently number one edible oil source globally. Among the conventional oil seed crops of India, soybean ranks third in its importance, next only to groundnut and rapeseed-mustard. Soybean seed is the biggest source of vegetable oil in the World. Soybean oil is used as edible oil for manufacturing of chocolates, ghees, soaps, paints, rubbers, lubricants, explosives, glycerins and antibiotics. There are several factors responsible for low seed yield of soybean. Among these the seed borne pathogens are one of the most important factors. Majority of seed borne diseases are caused due to fungi. Seed borne pathogens causes losses in terms of seed quality and quantity in soybean crop. The microflora also reduces the germination and seedling vigour index of soybean. They are responsible for seed rot, seedling blight, bacterial pustules, root / stem rot, foliar infection as well as pod blight diseases. (Agarwal *et. al.*,1974). One of the important

basic needs for higher agricultural production is quality seed which is characterized by high viability and vigour. To increase the production of soybean qualitatively and quantitatively, farmer requires healthy quality seeds with high percentage of germination and purity. Hence, it is imperative that seed must be tested before they are sown in the field. Seed health testing methods like agar plate methods and modified PDA method have been employed for detection of internal and external seedborne mycoflora of soybean, Solanke *et al*. (1997), Paul (1989) and Rajeswari and Meena Kumari, (2009). Considering these issues, present study was planned and conducted with the aim to detect and determine frequency of various seedborne fungi of soybean.

Materials and Methods:

A total of 5 varieties of soybean seed were collected Oilseeds Research Station, Latur and Seed Processing Unit (National Seed Project) VNMKV, Parbhani. The seeds were collected in polythene bags and stored at room temperature of 25±20C.

In Standard agar plate method Four hundred each seeds of soybean var. MAUS-71, MAUS-158, MAUS-162, MAUS-612 and JS-335 were placed at the rate of (10 seeds / Petriplate) containing 20 ml of two per cent water agar and incubated at 27±2^{0}C, for 7 days. After seven days of incubation, these plates were observed under stereo-binocular microscope to ascertain growth of various fungi associated with seeds of soybean. Based on cultural characters, various fungi appeared in Petriplates were aseptically isolated individually onto autoclaved and cooled PDA medium in separate Petriplates and incubated further for a week. After a week of incubation, well developed fungal colonies appeared. By applying hyphal tip technique, these fungi were transferred aseptically onto autoclaved and cooled PDA slants in test tube, incubated to proliferate and stored in refrigerator for further studies.

In modified PDA method Four hundred each seeds of soybean var. MAUS-71, MAUS-158, MAUS-162, MAUS-612 and JS-335 were placed at the rate of (10 seeds / Petriplate) containing 20 ml of autoclaved and cooled acidified Potato Dextrose Agar (pH 4.5). Seeds were placed after pre-treatment with 2-3% sodium hypochlorite solution for 3 to 5 minutes, washed in three sequential changes of sterile distilled water and the plates were incubated at 26±2^{0}C, for a week. After a week of incubation, the fungal colony growth was examined under stereo-binocular microscope.

Result and Discussion:

The analysis 5 varieties of soybean using standard agar plate methods and modified PDA method showed the association of six fungal species. The fungi detected were identified based on their cultural and morphological characteristics. The fungal species detected through standard agar plate methods and modified PDA method includes *Fusarium verticillioides*, *Macrophomina phaseolina*, *Alternaria alternata*, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer* (Table 1and 2) .

Per cent infectivity of seed mycoflora varied across the methods adopted and varieties tested. In standard agar plate method (Table 1 and Fig.1) germination per cent varied from 40.00 to 72.50%. In all the cultivars maximum seed germination 72.50% was observed in MAUS-71 and minimum seed germination 40.00% was observed in JS-335. The frequency (Table 1 and Fig.1) of association /incidence of all the six fungi was found maximum (100, 100, 100, 100 and 100%) and healthy seeds were absent (00.00%) in the seeds of all cultivars of soybean *viz.*, MAUS-71, MAUS-158, MAUS-162, MAUS-612 and JS-335.

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The results from Fig. 1 revealed that, among all the cultivars, highest frequency of various seed mycoflora was observed in MAUS-71 i.e., 38.00 and 32.00% of *F. verticillioides* and *M. phaseolina*, respectively. However, among these six fungi comparatively maximum frequency of *F. verticillioides* was observed. Among all the mycoflora associated, higher frequency was observed of *F. verticillioides* (avg. 27.45%), followed by *A. niger* (avg. 25.40%), *M. phaseolina* (20.25%) and very less frequency was of *R. stolonifer* (avg. 2.00%). Results of the present studies were in consonance with those reports earlier by the several workers (Lokesh and Hiremath 1992; Singh *et al*. 2003; Nasreen 2003; Ramesh *et al*. 2013). Lokesh and Hiremath (1992) reported that, agar plate method was found efficient in enumeration of seed mycoflora of pigeonpea. Singh *et al*. (2003) isolated eight different fungi of pearl millet seed sample by using agar plate method. Nasreen (2003) isolated 39 species of fungi in soybean seeds by using agar plate method. Ramesh *et al*., (2013) isolated 11 fungi from soybean seeds by agar plate method.

In modified PDA method Results (Table 2 and Fig.2) revealed that, in modified PDA method germination per cent was ranged between 67.50 to 95.00% and healthy seeds ranged from 8.75 to 42.50% in all the cultivars. The results (Table 2 Fig. 2) revealed that, among these six fungi comparatively maximum frequency of *F. verticillioides* was observed i.e., 25.00, 22.50, 22.50, 15.00 and 11.25% in cultivars MAUS-162, MAUS-71, MAUS-612, MAUS-158 and JS-335, respectively, followed by the fungi *viz*., *M. phaseolina* i.e., 25.00, 20.00, 15.00, 10.00 and 5.00% in cultivars MAUS-71, MAUS-612, JS-335, MAUS-162 and MAUS-158, respectively, *A. alternata* i.e., 20.00, 17.50, 12.50, 10.00 and 7.50% in cultivars JS-335, MAUS-612, MAUS-158, MAUS-71 and MAUS-162, respectively, *R. stolonifer* i.e., 20.00, 17.50 and 15.00% in cultivars JS-335, MAUS-158 and MAUS-162, respectively, *A. niger* i.e., 17.50, 10.00, 8.75, 7.50 and 5.00% in cultivars JS-335, MAUS-158, MAUS-71, MAUS-612 and MAUS-162, respectively, *A. flavus* i.e., 11.25, 7.50, 2.50, 2.50 and 1.25% in cultivars MAUS-162, JS-335, MAUS-158 MAUS-612 and MAUS-71, respectively.

In modified PDA method highest frequency was observed of *F. verticillioides* (avg. 19.25%), followed by *M. phaseolina* (avg. 15.00%) and *A. alternata* (avg. 13.50%) and very less frequency was of *A. flavus* (avg. 5.00%). Similar results were found by Mandhare *et al*. (2009). Mandhare *et al*., (2009) detected seed borne infection of *M. phaseolina* causing charcoal rot of soybean by using modified agar plate method.

However, higher per cent of the major six seedborne fungi were evidence in Agar plate method than modified PDA method.

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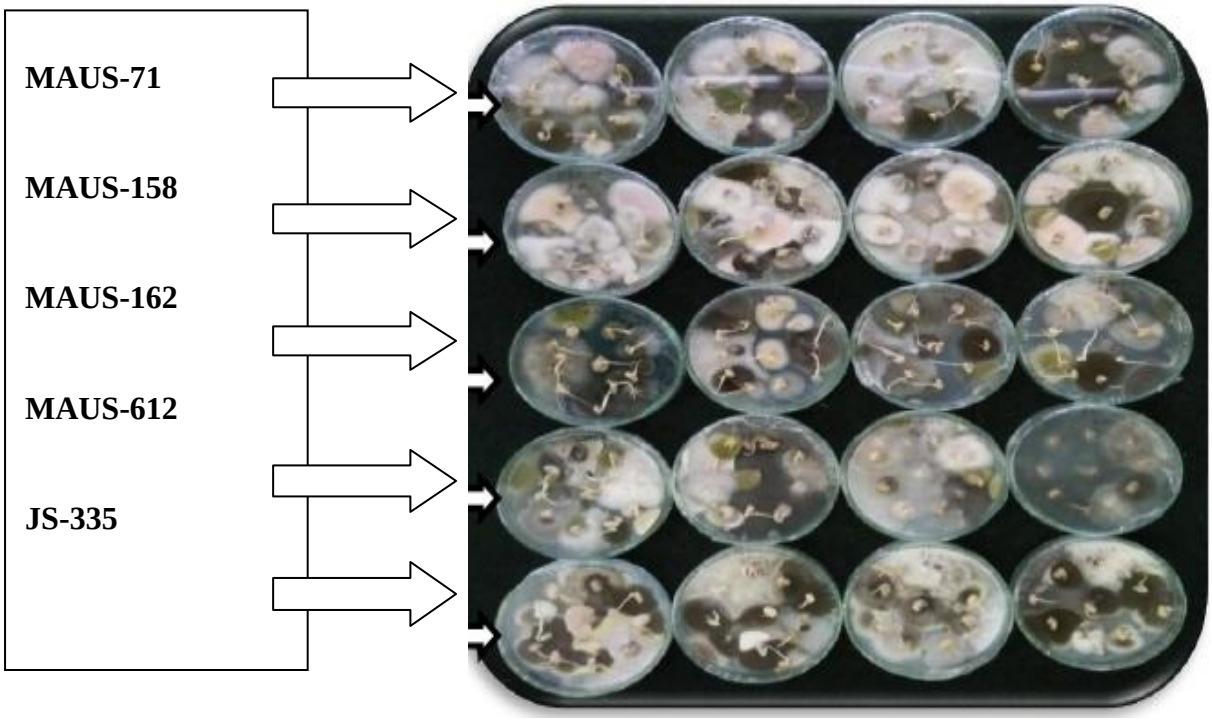

Fig. 1. Detection of Seedborn Mycoflora by Standard Agar Plate Method In Different Cultivars.

Table 1. Per cent frequency of various fungi associated with soybean seeds, By Standard agar plate method

Sr. No.	Genotype	Germination(%)	Healthy seeds (%)	Infected seeds (%)	Seed mycoflora (%)					
					F. verticillioides	*M. phaseolina*	*A. alternata*	*A. niger*	*A. flavus*	*R. stolonifer*
1.	MAUS-71	72.50	00	100	38.00	32.00	15.00	10.00	5.00	0.00
2.	MAUS-158	70.00	00	100	26.00	15.00	11.50	36.50	8.00	3.00
3.	MAUS-162	65.50	00	100	28.10	19.75	17.50	20.00	12.0	2.00
4.	MAUS-612	50.00	00	100	29.50	22.00	18.00	15.50	10.0	5.00
5.	JS-335	40.00	00	100	15.00	12.50	7.50	45.00	20.0	0.00
Mean		59.60	00	100	27.45	20.25	13.90	25.40	11.0	2.00

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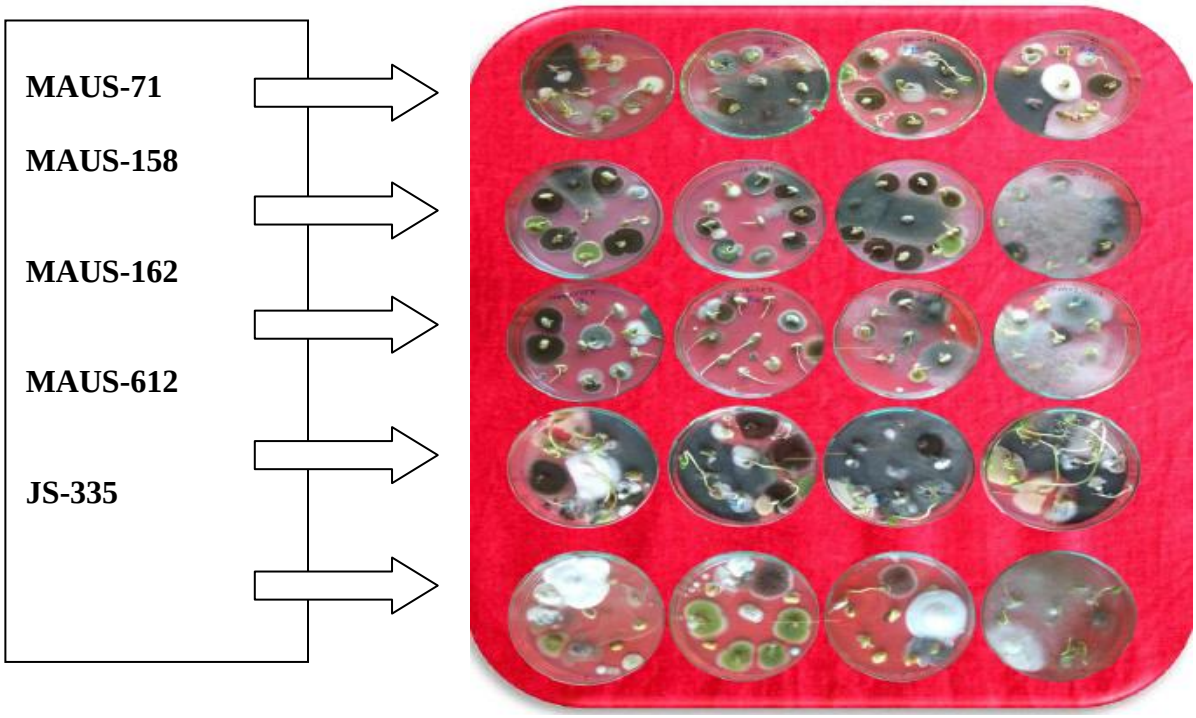

Fig. 2. Detection of Seedborn Mycoflora by Modified PDA Method In Different Cultivars.

Table 2. Per cent frequency of various fungi associated with soybean seeds, by Modified P.D.A. method

Sr. No.	Genotype	Germination %	Healthy seeds (%)	Infected seeds (%)	Seed mycoflora (%)					
					F. verticillioides	*M. phaseolina*	*A. alternata*	*A. niger*	*A. flavus*	*R. stolonifer*
1.	MAUS-71	15.00	11.25	88.75	27.50	2.50	6.25	12.50	37.50	2.50
2.	MAUS-158	16.00	35.00	65.00	25.00	5.00	7.50	5.00	12.50	10.0
3.	MAUS-162	20.00	37.50	62.50	30.00	12.50	10.00	2.50	2.50	5.00
4.	MAUS-612	18.00	28.50	71.50	32.50	10.50	7.50	5.00	7.50	8.50
5.	JS-335	16.00	5.00	95.0	28.00	3.00	8.25	15.00	35.75	5.00
Mean		17.00	23.45	76.55	28.50	6.70	7.90	8.00	19.15	6.20

References:

1. Afzal, R., Mughal, S.M., Munir, M., Sultana, K., Qureshi, R., Arshad, M. and Laghari, M.K.(2010). Mycoflora associated with seeds of different sunflower cultivars and its management. *Pak. J. of Bot*. 42 **(1)**: 435–445.
2. Agarwal, V.K., O.V. Singh and Y.L. Nene, (1974). Influence of fungicidal seed treatment on the mycoflora of stored seed and seedling emergence. *Indian J. Agric. Sci*., 43**(8)**: 820-824.
3. Deshmukh, A. M. and Kare, M. A. (2010). Study of seed mycoflora of some oilseed crops. *BIOINFOLET* 7**(4)**: 295-297.
4. Kakde, R. B. and Chavan, A. M. (2012). Nutritional changes in soybean and safflower oil due to storage fungi. *Current Botany* 3**(4)**: 18-23.
5. Lokesh, M.S. and Hiremath, R.V. (1992). Studies on seed mycoflora of redgram (*Cajanus cajan* L. Mill sp.) *Karnataka J. of Agri. Sci*. 5**(4)**:353-356.
6. Mandhare, V. K., Gawade, S. B and Suryawanshi, A. V. (2009). Detection and transmission of seed borne infection of *Macrophomina phaseolina* causing Charcoal rot in soybean. *J. Pl. Dis. Sci*. 4**(1)**: 130-131.
7. Mandhare, V. K., Gawade, S. B and Suryawanshi, A. V. (2009). Detection and transmission of seed borne infection of *Macrophomina phaseolina* causing Charcoal rot in soybean. *J. Pl. Dis. Sci*. 4**(1)**: 130-131.
8. Nasreen, N. (2003). Detecting Seedborne fungi of soybean by different incubation methods. *J. of Pl. Patho.* 01. pathogens and effect of fungicides. *J. of Agril. University*, 22 **(2):**168-170.
9. Paul, Y.S. (1989). Seedborne mycoflora of soybean and its control in Himachal Pradesh. *J.Myco. and Pl. Patho*. 19 **(3)**: 253-257.
10. Pradhan, A., Lakpale, N. and Khare, N. (2014) Effect of fungicidal seed treatment on seed borne mycoflora and seedling vigour of pigeon pea (*Cajanus cajan* L.) millsp. *J. Mycol. Pl. Pathol*. 44**(4)**: 447-449.
11. Radha, P. L., Papireddy, M., Madhu, B.M., Nagaraja, H., Gowda, A. N. S., Jyothi, G. and Chattannavar, S. N. (2015). Collection of seed samples from different sesame growing districts of northern Karnataka and initial seed health testing by standard blotter method. *Trends in Biosciences.* 8**(9)**: 2359-2371.
12. Rajeswari, B. and Meena Kumari, K.V.S. (2009). Bioagents and fungicides for the manage ment of seed and seedling diseases of soybean. *Ind. J. of Pl. Prot.* 37**(2)**: 121-131.
13. Ramesh, B.V., Hiremath, S.V., Naik, M.K., Amaresh, Y.S., Lokesh, B.K. and Vasudevan, S.N. (2013). Study of seed mycoflora of soybean from north eastern Karnataka. *J. of Agri. Sci*.,26 **(1)**: 58-62.
14. Singh, S. D., Swami, S. D. and Rawal, P. (2003). Seed mycoflora of pearl millet (*Pennisetum glaucum*) and its control. *Pl. Dis. Res.* 18**(2)**: 115-118.
15. Solanke, R.B., Kore, S.S and Sudewad, S.M. (1997). Detection of soybean seedborne pathogens and effect of fungicides. *J. of Agril. University*, 22 **(2):**168-170.