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Detection of Seed Mycoflora Associated with Indian Mustard (*Brassica juncea* L.) by Various Seed Health Testing Methods

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

India is one of the major oilseed crops grower. Mustard (*Brassica juncea* L.) is an important oilseed crop second after groundnut. Mustard is majorly grown in India which carry many seedborne diseases which causes not only seed rot, seedling blight but also reduction in quality of seed parameters which results qualitative and quantitative yield losses. In standard agar plate seed germination per centage ranged from 78.50 to 85.00% and in Modified PDA method ranged from

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70.00 to 78.00%. In standard agar plate and Modified PDA method highest per cent of infected seed was observed in Pusa bold 74.25% and 64.50%, respectively. In two seed health testing methods (SHT) were evaluated standard agar plate show higher seed mycoflora than modified PDA method. All two methods were found effective and reliable for the detection of seedborne fungi such as *Alternaria brassicae, Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Rhizoctonia solani, Penicillium* sp. and *Rhizopus stolonifer* isolated from mustard seeds of varieties Krishna, Kranti, Local and Pusa bold.

Keywords: Mustard; seed mycoflora; standard agar plate method; modified PDA method.

1. INTRODUCTION

Indian mustard (*Brassica juncea*. L.) belonging to the family Brassicaceae (Cruciferae) is one of the important oilseed crops grown in India. Mustard is globally used as oilseed, vegetable and condiments [1]. Area, production and productivity of rapeseed-mustard in the world is 36.59 (Mha) area, 72.37 (Mt) production and 1980 kg/ha productivity [2]. Indian mustard cultivation has occupied about 85 to 90% of total area under cultivation of mustard-rapeseed [3]. Rajasthan ranks first both in area and production of rapeseed mustard in the country which contributing 40% area and 44.97% production of India in 2021-22 [4]. There are so many constraints in mustard production like traditional land races, biotic stresses like diseases, insect pests, abiotic factors. Among these factors, disease is the most important yield reducing factor. Large number of diseases of mustard caused by fungal, bacterial and viral pathogens. Seed borne fungi reported to reduce germination which alter physiochemical properties of the seeds during storage, losses of the seed weight, germination potential, medicinal properties and discoloration, causing the losses to the extent of 24 per cent [5]. Seeds of mustard are known to carry several seed-borne fungi are *Alternaria* sp., *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* species, and *Rhizopus stolonifer* [6]. Seed health testing methods like agar plate and modified PDA methods have been employed for detection seed borne mycoflora. With the help of seed health testing methods, the sources of seedborne infections, location of pathogens within seed tissues can be identified. Detection is a first line approach in managing seedborne diseases of plants [7]. It is important to know the presence and identify seed mycoflora which damaging effect on mustard during crop production and storage period. With regards to seed yield losses, present study was planned and conducted with the aim to detect and determine frequency of various seedborne fungi of mustard.

2. MATERIALS AND METHODS

A total of four varieties of mustard seeds were collected from Local market and farmers from Latur district. For detecting seed borne mycoflora, the seed health testing procedures as prescribed by International Seed Testing Association [8] were followed for estimating incidence of mycotic genera from mustard seed were tested by Agar plate method and Modified PDA method respectively.

2.1 Standard Agar Plate Method

Four hundred seeds of each mustard var. Krishna, Kranti, Local and Pusa bold were used. Unsterilized seeds were placed @ 10 seeds / petri plate containing 20 ml of PDA medium and incubated at 27 ± 2^0C, for a week as described under. In this method uses nonsterilized seeds which showed both internal and external mycoflora. After a week of incubation, characteristics of fungal colonies from top and reverse were examined under stereo-binocular microscope.

2.2 Modified PDA Method

Four hundred seeds of each mustard var. Krishna, Kranti, Local and Pusa bold were used. Seeds were placed @ 10 seeds / petri plate containing 20 ml of autoclaved and cooled acidified Potato Dextrose Agar (pH 4.5). Seeds were placed after pretreatment 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 27 ± 2^0C, for a week. In this method uses sterilized seeds which showed only internal seed mycoflora. After a week of incubation, the fungal colony growth was examined under stereo-binocular microscope. Per cent of incidence of each fungus was recorded.

3. RESULTS AND DISCUSSION

The analysis four varieties of mustard using standard agar plate methods and modified PDA method showed the association of seven fungal species. The fungi detected were identified based on their morphological and cultural characteristics. The fungal species detected through standard agar plate methods and modified PDA method *viz*., *Alternaria brassicae, Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Rhizoctonia solani, Penicillium* sp. and *Rhizopus stolonifera* isolated from mustard seeds of varieties Krishna, Kranti, Local and Pusa bold (Tables 1 and 2).

3.1 Identification of Seed Mycoflora

The isolated seed mycoflora were purified by single spore isolation technique [9]. Thus, pure cultures obtained were maintained on potato dextrose agar slants. Total seven fungi isolated from mustard seeds *viz*., *Alternaria brassicae*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Rhizoctonia solani*, *Penicilium* sp*.* and *Rhizopus stolonifer* were characterized and identified on the basis of growth habit, cultural and morphological characters. Cultural characteristics *viz*., colony growth, colony morphology, colony colour, mycelial growth, conidia and sporangia observed under microscope etc. were considered.

1) **In Standard agar plate method,** the result (Table 1) revealed that, seed germination per centage ranged from 78.50 to 85.00%. However, it was highest in var. Krishna (85.00%) and it was lowest in var. Pusa bold (78.50%). Maximum healthy seeds were recorded in var. Krishna (45.00%) and it was minimum in var. Pusa bold (25.75%). The highest per cent of infected seed was observed in Pusa bold (74.25%) followed by Local (71.25%), Kranti (70.25%). However, it was lowest in var. Krishna (53.00%). Results also revealed that, per cent infection frequency of *Penicillium* sp was highest (13.75%), followed by *Fusarium oxysporum* (13.68%), *A. niger* (12.12%), *Rhizoctonia solani* (8.62%), *A. flavus* (8.00%), *A. brassicae* (5.87%) and *Rhizopus stolonifer* (5.12%). Dhawan et al. [10] used Standard agar plate methods and modified PDA method for for detection of six fungal genera including *Fusarium verticillioides*, *Macrophomina phaseolina*, *Alternaria alternata*, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer* from soybean seeds and reported that, per cent infection frequency of *Fusarium* sp. (27.36%) was highest in standard agar plate method and this method found to be superior method for detection of seedborne fungi in their study. Similar results were also found by Srinivas et al. [11], Ghosh et al. [12].

2) **In Modified PDA method,** the result (Table 2) showed that, seed germination per centage ranged from 70.00 to 78.00%. However, it was highest in Pusa bold variety (78.00%) and it was minimum in variety Krishna (70.00%). Maximum healthy seeds were recorded in var. Krishna (46.50%) and it was minimum in var. Pusa bold (35.50%). Result also revealed that, the highest per cent of infected seeds were recorded in Pusa bold (64.50%). However, least infected seeds were observed in var. Krishna (53.50%). the results also showed that, per cent infection frequency of *Fusarium oxysporum* was highest (13.87%), followed by *Penicillium sp* (13.12%), *A. niger* (12.37%), *Rhizoctonia solani* (6.00%), *A. flavus* (5.56%), *Rhizopus stolonifer* (4.87%) and *A. brassicae* (2.37%). The results of present study are similar with several earlier workers. Debbarama and Banik. [13] reported that, per cent infection frequency was highest *Penicillium* sp (14.67%) and *Aspergillus* sp (12.67%) as compared to other mycoflora and this modified PDA method was found superior to blotter paper method and water agar method. Meena et al*.* [14] detected 16 saprophytic as well as parasitic mycoflora of mustard. Among 16, 12 fungal species were reported by using modified PDA method and recorded that, per cent infection frequency was highest on *Fusarium* sp (2.05%) as compared to other mycoflora. Similar findings were in agreement with earlier workers Qumberani [15], Siddiqui [16], Bhajbhuje [17].

Table 1. Per cent frequency of various seedborne mycoflora of mustard seed isolated by Agar plate method

Sr. No.	Varieties	Germination (%)	Healthy seeds (%)	Infected seeds (%)	Seed Mycoflora (%)						
					Alternaria brassicae	*Fusarium oxysporum*	*Aspergillus niger*	*Asperegillus flavus*	*Rhizoctonia solani*	*Penicillium sp.*	*Rhizopus stolonifer*
1.	**Krishna**	85.00	45.00	53.00	5.00	12.00	6.00	4.50	7.00	14.00	4.50
2	**Kranti**	82.00	29.75	70.25	8.00	15.00	8.50	7.75	8.50	16.00	6.50
3	**Local**	80.00	28.75	71.25	6.50	13.50	12.50	7.75	9.00	16.50	5.50
4	**Pusa bold**	78.50	25.75	74.25	4.00	14.25	21.50	12.00	10.00	8.50	4.00
Mean		**81.37**	**32.31**	**67.18**	**5.87**	**13.68**	**12.12**	**8.00**	**8.62**	**13.75**	**5.12**

Table 2. Per cent frequency of various seedborne mycoflora of mustard seed isolated by modified PDA method

Sr. No.	Varieties	Germination (%)	Healthy seeds (%)	Infected seeds (%)	Seed Mycoflora (%)						
					Alternaria brassicae	*Fusarium oxysporum*	*Aspergillus niger*	*Asperegillus flavus*	*Rhizoctonia solani*	*Penicillium sp.*	*Rhizopus stolonifer*
1.	**Krishna**	70.00	46.50	53.50	2.50	13.50	4.00	5.50	4.50	16.00	7.50
2	**Kranti**	75.00	44.50	55.50	2.00	12.50	7.50	8.00	9.50	12.00	4.00
3	**Local**	76.00	40.75	59.25	2.00	14.00	17.00	5.75	6.00	9.50	5.00
4	**Pusa bold**	78.00	35.50	64.50	3.00	15.50	21.00	3.00	4.00	15.00	3.00
Mean		**73.50**	**41.81**	**58.18**	**2.37**	**13.87**	**12.37**	**5.56**	**6.00**	**13.12**	**4.87**

4. CONCLUSION

Seven major fungi *viz*., *Alternaria brassicae*, *Fusarium oxysporum*, *Aspergillus niger, Aspergillus flavus, Rhizoctonia solani*, *Penicillium* sp*.* and *Rhizopus stolonifer* were isolated from mustard varieties *viz*., Krishna, Kranti, Local and Pusa bold. The seed borne fungi associated with seed show poor germination and viability of seed. Mycoflora associated with seeds at the stage of storage which makes unfit for consumption and sowing. Identification and detection of pathogen is important for managing the seed borne diseases. In two seed health testing methods (SHT) were employed agar plate showed more seed mycoflora than modified PDA which found efficient. Modified PDA method was found to be most suitable for detection of *Fusarium oxysporum* in all the varieties. Among the four varieties of mustard *viz*., Krishna, Kranti, Local and Pusa bold, highest germination percent was observed in Pusa bold while it was minimum in var. Local. Highest seed infection was recorded in var. Pusa bold and minimum in var. Krishna.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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