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In Vitro Association of Seed Borne Fungi with Pea (*Pisum sativum*) in Central India

Sanjay Kharte*[1], Pramod Kumar Gupta[2], Yogita Gharde[3] and A K Singh[4]
Department of Plant Pathology
Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, 482004, (Madhya Pradesh), India

ABSTRACT

Pea is a most important legume in several developing and developed countries including India. In the present investigation, keeping in view these facts, Seed samples were collected from four districts of Madhya Pradesh *viz*. Katni, Mandla, Narsinghpur and Jabalpur for evaluation of associated fungi on pea seeds. These samples were studied for detection of seed mycoflora by Standard Blotter Paper Method recommended by ISTA. A total of six genera were identified and recorded from seeds of pea. During the surveyed years, the highest incidence of seed/seedling disease (53.0 and 32.4%) was recorded in Jabalpur, followed by Fusarium wilt (42.5 and 44.5%), powdery mildew (62.8 and 64.4%) and downy mildew (22.2 and 16.4%). The result of the present studies provides the baseline information about seed mycoflora for further studies and management of seed borne diseases associated with pea seeds.

Keywords: Disease, Fungi ,Legume,Mycoflora, Pea, Seed.

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the most important legume crops in India which belongs to the family (*Fabaceae)* Leguminosae (Brar *et al,* 2018; Pande and Kumar, 2024). Pea is generally sown in *Rabi* season in India from the beginning of October to mid of November in the plains (Falloon and Viljanen, 2001). Pea is an important grain legume in Asia and being a rich and cheap source of protein can help people improve the nutritional quality of their diets. It provides a nutritious meal rich in protein as 100 g of dried grains contain 1.8 g of fat, 62.1g of carbohydrates, 22.5 g of protein, 0.15 g of riboflavin, 0.72 mg of thiamine, 2.4 mg of niacin, 64 mg of calcium and 4.8 mg of iron (Rani *et al,* 2023). More specifically, the field pea is naturally rich in iron and zinc and, thus, can address two of the most common micronutrient deficiencies in the world (Amarakoon *et al,* 2012). Economically, it is a predominant export and cash crop in world trade and represents about 40% of the total trading in pulses (Oram and Agcaoili, 1994). In India, pea is grown in winter as well as summer seasons and each pea pod having several seeds of green or yellow colour. The area and production of green peas in India is about 5,46,000 ha and 5.45 million tonnes, respectively (NHB, 2017).

Pea production is challenged by a wide range of plant pathogens. Among the different diseases of pea *viz*., seed and seedling diseases, fusarium wilt, collar Rot, powdery mildew and downy mildew is a major limiting factor in its production and productivity (Kharte *et al,* 2021; Kharte *et al,* 2022a). However, the yield is influenced by the number of seed, soil-borne and foliar diseases. Moreover, the pea is the most sensitive to damping off, fusarium wilt, root rots, collar rot, powdery mildew, downy mildew and other foliar diseases causing heavy losses from seedling to pod filling stages and disease severity depends upon the abiotic factors (Hagedorn 1991; Kharte *et al,* 2022b). The association of fungi adversely affects the quality and health of the seeds. The term "seed mycoflora of seed borne fungi" is used for both qualitative as well as quantitative analysis of fungi occurring on or in the seeds (Neergaard, 1973). The seed mycoflora are carried over from year to year and from one place to another with the seeds which serve as primary source of infection for subsequent crops (Bhale *et al,* 2001). The isolated fungi were identified with the help of the monograph provided by (Domsch *et al,* 1980; Nelson *et*

Corresponding Author's Email - skpatho@jnkvv.org
1Department of Plant Pathology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, 482004, (Madhya Pradesh), India
2,4Krishi Vigyan Kendra, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, 482004, (Madhya Pradesh), India
3ICAR-Directorate of Weed Research, Jabalpur, 482004, (Madhya Pradesh), India

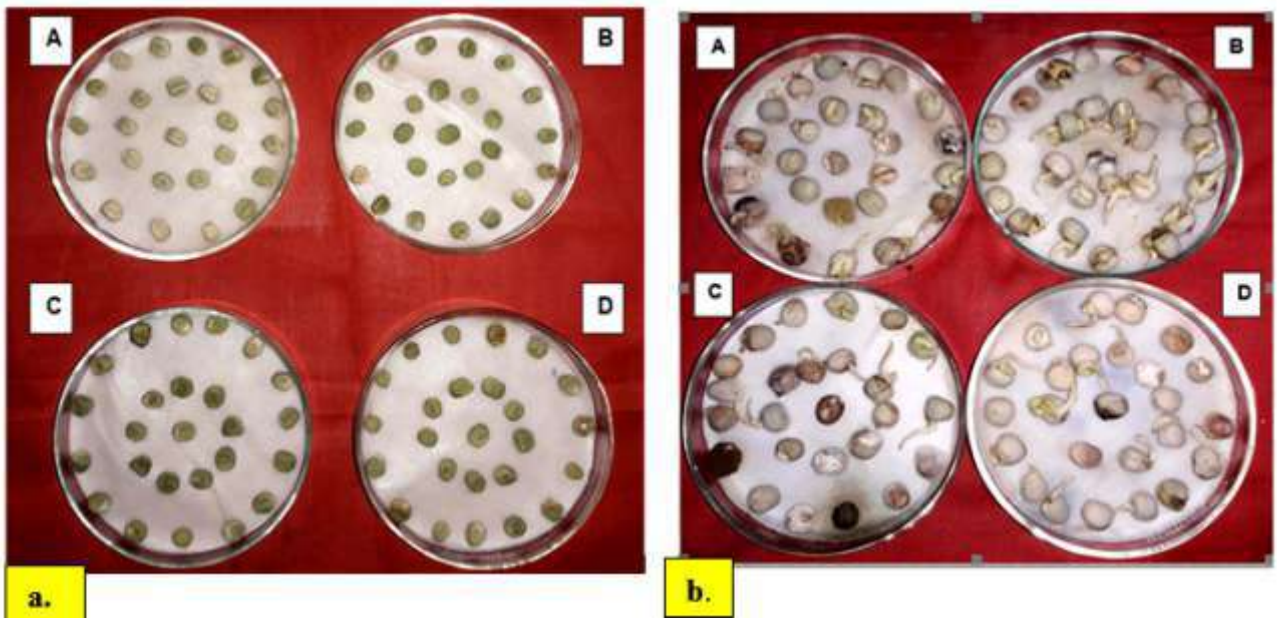

Fig. 1: a) Healthy seeds of pea and b) Association of fungi on pea seeds

al, 1983; Alexopolus *et al,* 2017) and authentic manuals (Neergaard and Mathur, 1980). The present study aims to identify the seed borne fungi associated with pea seeds under laboratory conditions, using standard blotter paper method. The effect of natural infection by fungi on pea seeds component and germination was also studied.

MATERIALS AND METHODS

Study location

The present investigation was conducted in the laboratory of the Department of Plant Pathology, JNKVV, Jabalpur, Madhya Pradesh. Seed samples of pea were collected from the four districts *viz*., Katni, Mandla, Narsinghpur and Jabalpur of Madhya Pradesh for identification of seed associated with mycoflora. Seed sample assessed at the Department of Plant Pathology for further studies.

Seed Mycoflora

Seed samples of peas were assessed by standard blotter paper method (ISTA, 1996), for the associated mycoflora. Three layers of blotter paper (Limonard, 1968, Lantos, 2002) cut into circular shape and 150 mm and 90 mm in diameter according to the size of Petri-plates were cut with the help of sterile scissor. The blotter paper was dipped & moistened with distilled water and extra water removed from the Petri-dish with the help of sterilized forceps. A total of 25 seeds were placed in such a manner that 15 seeds in 1st outer circle, nine seeds in 2nd circle and one in the center. The seeded plates were BOD-incubated for seven days at temperature of 26±2 °C. Seed were examined on 3rd Days, 5th days and 7th days After incubation (ISTA, 2005), seed mycoflora was recorded after one week by observing fungal growth on seeds under stereo, binocular microscope. Further, the fungal species were confirmed by preparing slides and their frequency of occurrence was expressed in percentage (Booth and Sutton, 1984)).

Per cent infection was assessed as suggested by Jha (1995) and the percent of each species was calculated as follows.

$$\text{Total fungal colonies (\%)} = \frac{\text{No. of seeds colonized in each plate by a particular fungal species}}{\text{Total number of seeds in each plate}} \times 100$$

RESULTS AND DISCUSSION

The association of seed associated fungi identified through blotter paper method showed great variability. A total of six fungal sp. was identified by standard blotter paper method *i.e., Aspergilus Niger*, *Alternaria alternata*, *Curvularia lunata, Fusarium oxysporum, Rhizopus stolonifer* and *Mucor* sp. were observed as seed associated fungi on pea seeds using standard blotter method in sterilized Petri plates. The prevalence of seed mycoflora on pea seed collected from four district of Madhya Pradesh *viz.,* Katni, Jabalpur, Mandla and Narsinghpur during 2018-19 (Table 1, Fig. 1), The maximum seed germination of 92% was recorded in Jabalpur district followed by 90%

Table 1. Association of seed mycoflora on pea seeds using Standard Blotter Paper Method

District	Seed Germination (%)	Percent seed mycoflora						
		Aspergilus niger	*Alternaria alternata*	*Curvularia lunata*	*Fusarium oxysporum*	*Rhizopus stolonifer*	*Mucor* sp.	Mean
Katni	90	3	5	4	8	4	1	4.16
Jabalpur	92	2	4	3	6	6	3	4.0
Mandla	80	5	4	2	12	0	1	4.0
Narsinghpur	84	4	6	4	8	2	0	4.0
Mean	86.5	3.5	4.75	3.25	8.5	3.0	1.25	

in Katni district. However, the minimum seed germination of 80% was recorded in Mandla district.

The maximum association of *Aspergilus niger* 05% was recorded in Mandla district followed by 04% in Narsinghpur district. However, the minimum association of 2% was recorded in Jabalpur district. The maximum association of *Alternaria alternata* 06% was recorded in Narsinghpur district followed by 05% in Katni district. However, the minimum association of 4% was recorded in Jabalpur and Mandla district. The maximum association of *Curvularia lunata* 04% was recorded in Narsinghpur and Katni district followed by 03% in Jabalpur district. However, the minimum association of 2% was recorded in Mandla district. The maximum association of *Mucor* sp. 03% was recorded in Jabalpur district followed by 01% in Katni and Mandla district. However, the minimum association of 0% was recorded in Narsinghpur district. The maximum association of *Rhizopus stolonifer* 06% was recorded in Jabalpur district followed by 04% in Katni district. However, the minimum association of 0% was recorded in Mandla district. The maximum association of *Fusarium* sp., 12% was recorded in Mandla district followed by 08% in Katni and Narsinghpur district. However, the minimum association of 6% was recorded in Jabalpur district.

The present investigation is inconsistent with Sheela (2015), she reported both methods and agar plate method as the most suitable technique for the detection of fungi. Though both methods remained conducive, higher frequency of fungi was recorded with the agar plate method. A total of 10 fungi were found associated with peas seeds, including 09 fungi viz; *Alternaria alternata*, *Aspergillus flavus*, *A. fumigates*, *A. niger*, *Curvularia lunata*, *F. oxysporum*, *Penicillium*, *Rhizopus stolonifer* and *Stemphylium* were isolated through blotter paper methods. Sidra *et al* (2019) determined the samples of 2016 and 2017, *A. alternata* had the highest frequency percent of seed mycoflora (28.33 and 36.66%), followed by *A. niger* (25 and 31.66%), *C. lunata* (20 and 10%), *R. stolonifer* (18.33 and 13.33%), *F. oxysporum* (15 and 11.66%), and *A. fumigates* (15 and 16.66%). were isolated through blotter paper methods. High incidence of *Alternaria sp., Fusarium sp., Aspergillus sp., Penicillium sp.* and *Rhizopus sp.* shows the economic importance of these mycoflora and confirms the findings of (Hagedorn, 1984). Ozgonen and Gulcu (2011), also determined mycoflora of pea seeds and found some most common fungi *viz., Fusarium* sp., *Alternaria* sp., *Macrophomina phaseolina*, *Phytophthora megasperma, Rhizoctonia solani* and *Sclerotium rolfsii* from peas seed.

CONCLUSION

The seed-borne pathogens can cause severe losses and reduce production of pea at different stages of the crop like, seed, seed germination, seedling, vegetative, flowering and budding stage. A total of six different fungal species belonging to different genera were isolated from different four districts of pea seeds through blotter paper that revealed the association of fungal pathogens with pea seeds. The results of the

present study provided the baseline information about seed mycoflora for further studies and management of seed-borne diseases associated with Pea seeds in Jabalpur division of Madhya Pradesh.

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