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ASSESSMENT OF SEED-ASSOCIATED MYCOFLORA IN FARMER-SAVED SOYBEAN AND GROUNDNUT SEEDS ACROSS DIFFERENT BLOCKS OF JABALPUR, INDIA

Govind Junjadia[1*], Ashish Kumar[2], Ramniwas Ghasal[3], Jyoti Jhirwal[3], and Megha Jaimini[3],
[1]Department of Plant Pathology, College of Agriculture, Jodhpur Agricultural University, Jodhpur, Rajasthan, India
[2]Department of Plant Pathology, College of Agriculture, Jabalpur Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (Madhya Pradesh), India
[3]Department of Plant Pathology, CoA, Agricultural University, Jodhpur, Rajasthan, India
*Corresponding author E-mail: govindchoudhary1644@gmail.com
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ABSTRACT

Seed borne pathogens, including bacteria, fungi and viruses, reside on or within seeds and pose significant risks for the transmission of diseases to subsequent crops. Seed-handling practices, such as allowing seeds to fall on nets or the ground during harvest, can increase the incidence of seedborne fungi. Additionally, improper storage conditions, particularly elevated moisture and suboptimal temperatures, facilitate fungal proliferation, while cool and damp nursery conditions at sowing further promote disease development. In this study, farmers' saved seed samples of soybean and groundnut were collected from 45 villages across seven blocks of Jabalpur district (Jabalpur, Patan, Panagar, Sihora, Majholi, Kundam and Shahpura). The collection comprised 24 soybean and 21 groundnut seed samples, all exhibiting varying degrees of seed deformation, discoloration, impurities and mechanical damage, likely reflecting suboptimal storage practices. Among soybean samples, the highest proportion of healthy seeds was recorded in Patan block, whereas the lowest was observed in Jabalpur block. Five fungal species namely *Macrophomina phaseolina*, *Fusarium oxysporum*, *Alternaria alternata*, *Aspergillus flavus* and *Aspergillus niger* were isolated, with the highest total fungal incidence in Shahpura and the lowest in Panagar. Groundnut seed samples exhibited maximum physical impurities in Jabalpur block and minimum in Majholi. Only two fungal species, *Aspergillus flavus* and *A. niger*, were detected in groundnut seeds, with highest prevalence in Kundam and lowest in Shahpura. This study highlights the widespread occurrence of seedborne fungi in farmer-saved soybean and groundnut seeds, emphasizing the need for improved harvesting, handling and storage practices to minimize pathogen transmission and ensure seed quality.

Keywords **:** Seed associated mycoflora, soybean, groundnut, *Aspergillus flavus, Macrophomina phaseolina*

Introduction

Seed represents one of the most critical inputs available to smallholder farmers and is universally recognized as the cornerstone of crop productivity (Biemond et al., 2013). Seedborne pathogens including bacteria, fungi and viruses reside either on the seed surface or within the embryo and have the potential to infect subsequent crops. Among these, fungal contamination is particularly significant, as it can directly and indirectly compromise seed quality. Direct effects include fungal colonization of the kernel and production of metabolites that alter grain composition or metabolism, rendering seeds unsuitable for human or animal consumption. Indirectly, contaminated seeds can lead to reduced germination, impaired seedling vigour and lower crop yield, ultimately threatening food security (Bishaw, 2004).

Seedborne fungal incidence is often exacerbated by certain harvesting and handling practices, such as allowing seeds to fall on nets or the ground before collection, as well as by improper storage conditions, particularly high moisture and suboptimal

temperatures. Cool and humid nursery conditions at sowing further favour fungal proliferation, promoting early disease development (Mehrotra and Aggarwal, 2003). In addition, infected seeds can serve as a source of pathogen introduction into previously uninfested soils, potentially establishing soilborne pathogens and expanding their geographic distribution (Neergaard, 1986). While the use of clean, disease-free seed is essential for disease management, the identification and maintenance of low-disease-pressure areas for seed production are increasingly challenging under changing environmental conditions. Consequently, integrated pre-harvest and post-harvest strategies-including careful irrigation management, timely drying, careful harvesting and thorough cleaning remain crucial to mitigate fungal contamination and preserve seed quality (Colley, 2010; Mashilla, 2004). Given these challenges, there is a growing need for integrated seed treatment strategies that combine chemical fungicides with beneficial biocontrol agents such as *Trichoderma* spp. and *Pseudomonas* spp. These agents not only suppress seedborne pathogens but also enhance plant growth and contribute to bioremediation of soils, thereby complementing the protective effects of fungicides (Kharte *et al.*, 2022; Kumar and Sahu, 2014, 2015; Kumar *et al.*, 2015). By evaluating the efficacy of both biocontrol agents and fungicidal treatments, it is possible to establish correlations between pathogen suppression, seed quality and seedling vigour, enabling the development of sustainable and multifunctional seed management strategies. However, to implement such strategies, it is crucial to identify the seed associated mycoflora present on the seed and accordingly mitigation strategies can be adopted. Soybean and groundnut being two important oil seed crops of Madhya Pradesh are vulnerable to various seed associated mycoflora and subsequently lead to reduced germination and poor emergence (Khare *et al.*, 2025a, 2025b, 2025c; Chouhan *et al.*, 2025). Therefore, in view of the critical impact of seed-associated pathogens on initial plant populations and subsequent crop productivity, the present study was undertaken to characterize the seed-associated mycoflora in soybean and groundnut to assess its effects on key seed quality parameters, providing a foundation for the development of integrated disease management strategies.

Materials and Methods

Collection of Seed Samples

Farmers saved seed samples of soybean and groundnut were collected from seven blocks of Jabalpur district, Madhya Pradesh: Jabalpur, Panagar, Kundam, Patan, Majholi, Shahpura and Sihora. The survey was conducted during September 2021 to assess the occurrence of seed-associated mycoflora and their impact on seed health and quality. From each block, representative seed samples were collected, assigned unique codes, packed in paper envelopes and stored in a deep freezer until analysis. Seed health testing procedures followed the guidelines of the International Seed Testing Association (ISTA, 1976).

Examination of Dry Seeds

Seed morphological quality was assessed following Agarwal and Sinclair (1987) with minor modifications. From each sample, 20 g of seeds were randomly selected and divided into four 5 g fractions. Each fraction was spread evenly in a Petri plate and inspected with a hand lens or stereobinocular microscope. Seeds were categorized as: Deformed seeds (e.g., shriveled), discolored seeds, damaged seeds (Mechanically damaged or Insect-damaged), impurities (Plant debris - leaf and pod fragments, Inert material- stones, sand etc.) and apparently healthy seeds. Seeds and impurities in each category were pooled, weighed on an electronic balance and expressed as percentage by weight.

Detection and Isolation of Seedborne Fungi

Standard Blotter Paper Method

External seedborne fungi were detected using the ISTA blotter method (Neergaard, 1979). Three layers of Whatman™ No. 1 filter paper were soaked in sterile distilled water, excess water drained and placed in 90 mm Petri dishes. Ten seeds per sample were placed equidistantly; a total of 400 seeds per crop were evaluated. Plates were incubated for 7 days at 20°C under an alternating 12 h light/12 h dark cycle using near-ultraviolet or fluorescent light. On the seventh day, seeds were examined under a stereobinocular microscope and fungi were identified based on conidia, conidiophores and fruiting structures. The frequency of each fungus was calculated as:

$$\frac{\text{No. of seeds containing a particular fungus}}{\text{Total seed observed}} \times 100$$

Paper Towel Method (Rolled Towel Method)

Seed germination, vigour and the impact of seedborne inoculum were assessed using the rolled paper towel method, following ISTA rules. Fifty seeds per sample were placed on two layers of moist germination paper (10 seeds per row), covered and gently rolled. Rolls were incubated in a seed germinator at 26°C in a slanted position for 7–10 days. First and final germination counts were recorded on days 7 and 10, respectively. Seedlings were inspected

daily for morphological normality, vigour and mortality over 168 h.

Seedling vigour was calculated following Abdul-Baki and Anderson (1973). Root length was measured from the collar to the primary root tip and shoot length from the collar to cotyledon attachment. The Seed Vigour Index (SVI) was calculated as:

Vigour index = Seedling Length (Shoot + Root Length) x germination percentage

Statistical Analysis

All experiments were conducted in triplicate and data were expressed as mean ± standard deviation. Differences among blocks and treatments were analysed using ANOVA and correlations between fungal incidence and seed quality parameters were determined to assess the impact of seed-associated mycoflora.

Results

Survey and detection of seed borne mycoflora of soybean and groundnut seeds

A comprehensive survey was conducted across seven blocks of Jabalpur district during 2021 to collect farmers saved seed samples of soybean and groundnut. In total, 45 composite seed samples were collected, comprising 24 soybean samples and 21 groundnut samples. Soybean samples were obtained from 24 villages, with a minimum of ten subsamples per village combined to form a single composite sample. Groundnut samples were collected from 21 villages across six blocks (Fig.1). The survey revealed widespread presence of seed-associated fungi in both crops, with varying levels of physical seed defects, including deformation, discoloration and mechanical or insect damage. Soybean samples from Patan block exhibited the highest proportion of apparently healthy seeds, whereas samples from Jabalpur block had the lowest healthy seed percentage, indicating variability in seed quality across blocks. Groundnut samples showed maximum physical impurities in Jabalpur and minimum in Majholi block.

Fungal isolation identified five dominant species in soybean seeds: *Macrophomina phaseolina*, *Fusarium oxysporum*, *Alternaria alternata*, *Aspergillus flavus* and *Aspergillus niger*. Among blocks, Shahpura exhibited the highest total fungal incidence, while Panagar had the lowest. In groundnut, two fungal species namely *Aspergillus flavus* and *A. niger* were detected, with Kundam showing the highest incidence and Shahpura the lowest. Overall, the survey confirmed the prevalence of seedborne fungi across all blocks, with clear block-wise differences in both seed health and fungal load, highlighting the critical need for effective seed treatment strategies to improve germination, seedling vigour and overall crop productivity.

Physical purity analysis of Farmer-Saved Soybean Seeds

Composite seed samples were prepared by pooling equal quantities of soybean seeds collected from each surveyed block of Jabalpur district. Dry seed evaluation was conducted on seven composite samples, each representing one block and seeds were classified into five quality categories based on physical characteristics. Marked variation in seed quality was observed across blocks. The highest proportion of deformed seeds was recorded in samples from Jabalpur block (12.00%), closely followed by Sihora (11.97%), whereas Majholi block exhibited the lowest incidence of deformation (5.50%). Discoloured seeds, predominantly purple–reddish in appearance, were most abundant in Jabalpur samples (16.94%), while Patan block recorded the lowest proportion of discoloured seeds (6.17%). Both insect-damaged and mechanically damaged seeds were detected in all composite samples when examined using a diaphanoscope. The incidence of insect damage ranged from 0.83 to 1.40%, whereas mechanically damaged seeds varied from 1.00 to 2.16% across blocks. In addition, impurities, including plant debris and inert materials, were present in all samples, although their proportions varied among locations (Table 1; Fig.2). The proportion of apparently healthy seeds differed significantly between blocks. The highest percentage of healthy seeds (81.57%) was recorded in soybean samples from Patan block, whereas the lowest percentage (64.12%) was observed in samples from Jabalpur block. Overall, the dry seed evaluation revealed substantial block-wise variability in physical seed quality, reflecting differences in harvesting, handling and storage practices among farmers and underscoring the potential risk of compromised seed performance in subsequent crop establishment.

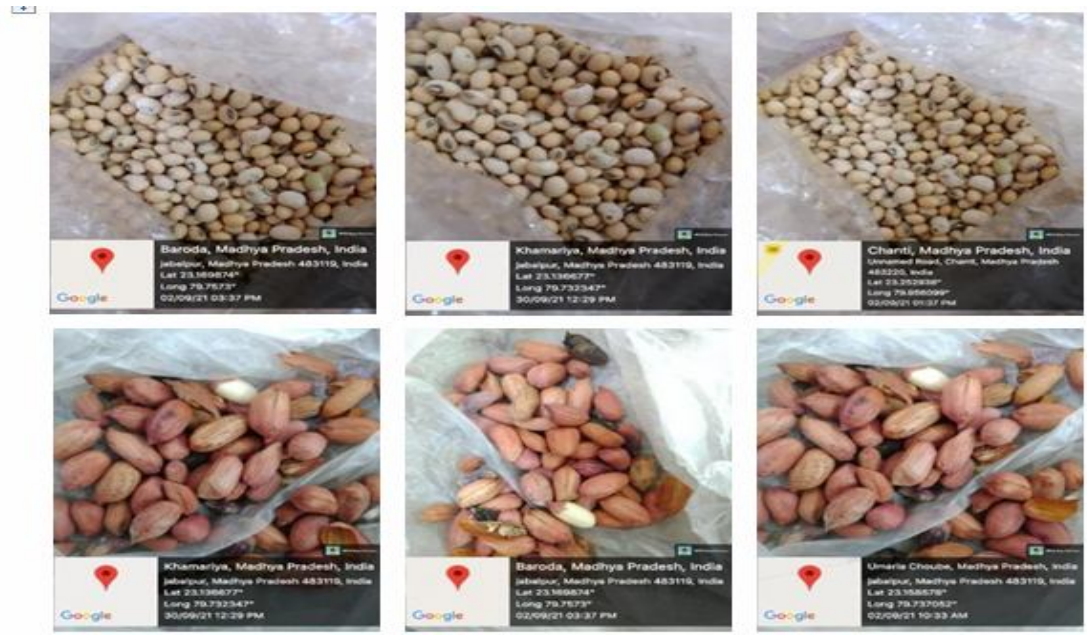

Fig. 1: Farmers saved seed sample of soybean and groundnut collected from different blocks of Jabalpur district

Fig. 2: Abnormalities and impurities in soybean (A) and Groundnut (B) farmers' saved seed samples

Table 1: Seed abnormalities and impurities in Farmers' saved soybean seeds samples

S. No.	Categories of seed impurities	Per cent category (by weight basis) per sample						
		Jabalpur	**Panagar**	**Kundam**	**Patan**	**Majholi**	**Shahpura**	**Sihora**
1	Deformed seeds							
	(i) Shrivelled	12	11.94	9.52	7.29	5.5	10.7	11.97
2	Discoloured seeds							
	(i) Purple Reddish	16.94	12.14	12.71	6.17	8.62	14.06	6.98
3	Damaged seeds							
	(i) Mechanically	2.16	1	1.31	1.22	1.48	1.13	1.13
	(ii) Insects	1.4	0.58	1	0.91	0.86	1.16	0.83
4	Impurities							
	(i) Plant debris	1.75	1.1	1	1.08	1.55	1.51	1.05
	(ii) Inert material	1.63	1.51	1.06	1.76	1.55	1	1.63
5	Apparently healthy seeds	64.12	71.73	73.4	81.57	80.44	70.44	76.41

Seed-Associated Mycoflora in Soybean Detected by the Standard Blotter Method

Seed health analysis using the standard blotter paper method revealed the presence of five fungal species associated with farmers' saved soybean seeds: *Macrophomina phaseolina*, *Fusarium oxysporum*, *Alternaria alternata*, *Aspergillus flavus* and *Aspergillus niger*. The frequency of occurrence of these fungi varied across blocks, ranging from 6.13–13.96% for *M. phaseolina*, 2.36–7.60% for *F. oxysporum*, 1.33–5.00% for *A. alternata*, 0.83–2.13% for *A. flavus* and 1.10–2.63% for *A. niger*. Among the surveyed blocks, the highest total fungal incidence on soybean seeds (29.02%) was recorded in samples collected from Shahpura block, whereas the lowest overall mycoflora incidence (14.88%) was observed in Panagar block (Table 2). Overall, *M. phaseolina* emerged as the most prevalent seed-associated fungus, followed by *F. oxysporum* and *A. alternata*, while *Aspergillus* species occurred at comparatively lower frequencies. These results indicate substantial block-wise variability in fungal load, reflecting differences in seed handling, storage conditions and local agro-environmental factors.

Table 2: Per cent incidence of mycoflora of soybean seeds detected by Standard Blotter Method

Block/ Mycoflora	Seed associated mycoflora (%)					Total mycoflora (%)
	M. phaseolina	***F. oxysporum***	***A. alternata***	***A. flavus***	***A. niger***	
Jabalpur	6.7	4.16	3.13	1.2	1.5	16.69
Panagar	6.2	2.36	2.43	1.26	2.63	14.88
Kundam	12.5	6.36	1.33	1.6	1.73	23.52
Patan	6.13	2.63	5.4	0.83	2.36	17.35
Majholi	9.23	4.4	5	2.13	1.36	22.12
Shahpura	13.76	7.6	4.63	1.93	1.1	29.02
Sihora	13.96	6.9	4.6	1.93	1.16	28.55
Mean	9.78	4.92	3.79	1.55	1.69	21.73

Detection of Seed-Associated Mycoflora in Soybean Using the Paper Towel Method

Seed health assessment using the paper towel method revealed that *Macrophomina phaseolina*, *Fusarium oxysporum*, *Alternaria alternata*, *Aspergillus flavus* and *Aspergillus niger* were the predominant seed-associated fungi in soybean across the surveyed blocks, occurring at variable frequencies. The overall incidence of seed-associated mycoflora detected by this method ranged from 0.85 to 9.17%. Among the identified fungi, *M. phaseolina* exhibited the highest frequency of occurrence, ranging from 2.93 to 9.17%, followed by *F. oxysporum* (1.64–5.56%) and *A. alternata* (1.36–3.91%). Lower frequencies were recorded for *A. flavus* (0.85–4.64%) and *A. niger* (0.95–1.61%). Block-wise analysis indicated that the maximum total fungal incidence (21.84%) was observed in soybean seed samples from Shahpura block, whereas the minimum incidence (11.45%) occurred in samples collected from Panagar block. These findings demonstrate pronounced spatial variability in seed-associated fungal communities under the paper towel assay (Table 3).

Table 3: Per cent incidence of mycoflora of soybean seeds detected by paper towel method.

Block/ Mycoflora	Seed associated mycoflora (%)					Total mycoflora (%)
	M. phaseolina	*F. oxysporum*	*A. alternata*	*A. flavus*	*A. niger*	
Jabalpur	4.35	2.98	1.48	2.64	1.23	12.68
Panagar	4.93	1.64	1.36	2	1.52	11.45
Kundam	8.42	3.86	1.67	2	1.25	17.2
Patan	3.3	2.04	3.91	0.85	1.61	11.71
Majholi	2.93	2.88	3.48	4.64	1.18	15.11
Shahpura	9.17	5.56	3.69	2.47	0.95	21.84
Sihora	8.16	4.85	2.89	1.79	1.02	18.71
Mean	5.89	3.4	2.64	2.34	1.25	15.53

Dry Seed Examination of Groundnut

Composite seed samples were prepared by pooling equal quantities of farmers' saved groundnut seeds collected from individual blocks, resulting in seven mixed samples representing the major groundnut-growing blocks of Jabalpur district. Dry seed examination classified each composite sample into five distinct categories based on physical seed quality attributes. The highest proportion of deformed seeds was recorded in samples from Sihora block (13.91%), closely followed by Jabalpur block (13.31%), whereas Majholi block exhibited the lowest incidence of deformation (5.36%). Seed discoloration, primarily manifested as blackish-white kernels, was most pronounced in samples from Jabalpur block (22.62%), while the lowest level of discoloration was observed in Sihora block (10.18%). Both insect-damaged and mechanically damaged seeds were detected across all samples, although their proportions varied among blocks. Additionally, impurities, consisting mainly of plant debris and inert matter, were present in all composite samples at variable levels. The highest proportion of presumably healthy seeds was recorded in the Majholi block (75.47%), indicating superior seed quality, whereas the lowest proportion was observed in Jabalpur block samples (57.09%), reflecting comparatively poor physical seed condition (Table 4).

Table 4: Seed abnormalities and impurities in different groundnut seeds samples

S. No.	Categories of seed impurities	Per cent category (by weight basis) per sample						
		Jabalpur	**Panagar**	**Kundam**	**Patan**	**Majholi**	**Shahpura**	**Sihora**
1	Deformed seeds							
	(i) Shrivelled	13.31	11.06	8.5	6.18	5.36	9.6	13.91
2	Discoloured seeds							
	(i) Blackish white	22.62	13.65	15.43	14.88	13.52	19.11	10.18
3	Damaged seeds							
	(i) Mechanically	2.16	1.74	1.59	1.5	1.37	1.23	1.06
	(ii) Insects	1.46	0.83	0.98	0.98	1.43	1.68	0.82
4	Impurities							
	(i) Plant debris	1.83	1.25	0.98	1.28	1.54	1.51	1.2
	(ii) Inert material	1.53	1.57	1.1	1.7	1.31	0.86	1.66
5	Apparently healthy seeds	57.09	69.9	71.42	73.48	75.47	66.01	71.17

Seed-borne Mycoflora of Groundnut

Standard Blotter Paper Method

Using the standard blotter paper method, two fungal species, *Aspergillus flavus* and *Aspergillus niger*, were consistently detected in farmers' saved groundnut seed samples collected from different blocks of Jabalpur district. The frequency of occurrence of *A. flavus* varied from 3.77 to 9.33% across the composite samples, whereas *A. niger* exhibited a broader range, with frequencies spanning 0.89 to 10.26%. The highest total incidence of seed-associated mycoflora was recorded in samples from Kundam block (15.26%), while the lowest overall fungal incidence was observed in samples from Shahpura block (6.66%), indicating substantial spatial variation in fungal contamination among blocks (Table 5).

Paper Towel Method

Consistent with the blotter assay, *Aspergillus flavus* and *Aspergillus niger* were also recovered using the paper towel method; however, the overall recovery of seed-associated fungi was comparatively lower. The frequency of *A. flavus* ranged from 2.50 to 6.00%,

while *A. niger* occurred at frequencies between 1.00 and 7.00% across the composite samples. The maximum total fungal incidence under the paper towel method was again recorded in seed samples from Kundam block (11.00%), whereas the minimum incidence was detected in samples from Shahpura block (4.67%), corroborating the block-specific trends observed in the blotter assay (Table 6).

Table 5: Per cent incidence of mycoflora of groundnut seeds detected by Standard Blotter Method

Block/Mycoflora	Seed associated mycoflora (%)		Total mycoflora (%)
	A. flavus	***A. niger***	
Jabalpur	5.57	5.08	10.65
Panagar	3.77	4.66	8.43
Kundam	5	10.26	15.26
Patan	8.51	4.62	13.13
Majholi	9.33	5.33	14.66
Shahpura	5.77	0.89	6.66
Sihora	6.11	2.63	8.74
Mean	6.29	4.78	11.08

Table 6: Per cent incidence of mycoflora of groundnut seeds detected under paper towel Method

Block/Mycoflora	Seed associated mycoflora (%)		Total mycoflora (%)
	A. flavus	***A. niger***	
Jabalpur	5.00	3.33	8.33
Panagar	2.50	3.00	5.50
Kundam	4.00	7.00	11.00
Patan	5.25	4.00	9.25
Majholi	6.00	4.33	10.33
Shahpura	3.67	1.00	4.67
Sihora	5.00	2.00	7.00
Mean	4.49	3.52	8.01

Discussion

The present study offers a systematic evaluation of seed health and seed-associated mycoflora in farmers saved soybean and groundnut seeds collected from seven agricultural blocks of Jabalpur district, Madhya Pradesh. Visual inspection and diaphanoscope-based analysis revealed a consistently high incidence of deformed, discoloured, shrivelled, mechanically damaged and insect-infested seeds across both crops. Such deterioration is most plausibly attributable to inadequate post-harvest handling and storage practices under conditions of high ambient humidity and poor aeration. Seeds exhibiting these defects are unsuitable for consumption or industrial use, resulting in significant economic losses, as previously reported by Anwar *et al*. (1995). Pronounced spatial variation in seed quality was evident among blocks. In soybean, the proportion of apparently healthy seeds ranged from 81.57% in Patan to 64.12% in Jabalpur, reflecting the influence of localized agronomic and storage environments. In addition to physical impurities, five fungal species namely *Macrophomina phaseolina*, *Fusarium oxysporum*, *Alternaria alternata*, *Aspergillus flavus* and *A. niger* were consistently detected using both standard blotter and paper towel methods, with the highest fungal incidence observed in Shahpura and the lowest in Panagar. Among these, *A. alternata*, *A. flavus* and *A. niger* act as opportunistic saprophytes capable of reducing germination and seedling vigour through enzymatic degradation of seed reserves and mycotoxin production. Seed-associated fungi constitute a major constraint to crop productivity, exceeding other pathogen groups in their overall economic impact (Paplomatas, 2006). Their detrimental effects on germination, biochemical composition and seed reserve mobilization are well documented (Anwar *et al*., 1995; Bhattacharya; Rana, 2002; Afzal *et al*., 2010; Kumar 2015; and Kharte *et al* 2022) and the present findings corroborate earlier reports on farmers saved soybean seeds (Hamane *et al*., 2020).

In groundnut, dry seed examination revealed widespread physical impurities across all blocks, with the highest deterioration recorded in Jabalpur and the lowest in Majholi. Consequently, the proportion of apparently healthy seeds ranged from 75.47% in

Majholi to 57.09% in Jabalpur. Mycological analysis consistently detected *A. flavus* and *A. niger*, with the highest fungal incidence in Kundam and the lowest in Shahpura, confirming the strong influence of storage conditions on fungal proliferation, in agreement with Kumar 2014; Adithya *et al*. (2017); Chouhan *et al.* (2025); Khare *et al.* (2025).

Overall, the study highlights seed-associated mycoflora and physical impurities as critical yet underappreciated constraints in farmers' saved seed systems. The close association between seed deterioration and fungal incidence underscores the urgent need for integrated seed health management strategies that combine improved storage practices with effective biological and chemical seed treatments to ensure seed quality, enhance crop establishment and minimize yield losses.

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