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Prevalence and Impact of Seed-borne Fungal Pathogens on Soybean Quality in Telangana

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ABSTRACT

The present investigation was conducted during *kharif,* 2021 (June-September) at PJTAU, Hyderabad, Telangana, India to study the impact of seed-borne fungal pathogens on seed health and quality of soybean. Soybean seed samples were collected from various locations in Telangana state, and subjected to standard blotter and agar plate methods for the detection of seed-borne mycoflora associated with soybean. A total of eight fungal species, belonging to six genera, were detected, including *Fusarium oxysporum*, *Fusarium semitectum*, *Macrophomina phaseolina*, *Colletotrichum truncatum*, *Curvularia* spp., *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* spp. using both the detection methods and among these, *F. oxysporum* and *M. phaseolina* were found to be predominant. The collected seed samples were also categorized into different grades based on visual observation, and the 100-seed weight was also recorded. To evaluate seed quality parameters, rolled paper towel method was employed. Among all the samples, the JS335 variety from Nizamabad district exhibited the highest total seed infection (49.02%), with the lowest germination (64.63%) and vigour index-I (1496). In contrast, the Basara variety from Adilabad district recorded the lowest seed infection (19.76%) with the highest germination (85.05%) and vigour index-I (3049). The results indicated a positive correlation between seed deformities, 100-seed weight and seed infection % in soybean, and these pathogenic seed-borne fungi were also found to be associated with reduced germination and other seed quality parameters.

KEYWORDS: Soybean, seed-borne mycoflora, isolation, seed infection, seed quality

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Soybean (*Glycine max* L.), popularly known as the "Golden bean" or "Wonder crop," is one of the most important legume crops globally. Originating in East Asia and belonging to the Fabaceae family, the soybean is cultivated extensively for food, feed, and industrial purposes. In India, it is the second-largest oilseed crop after groundnut. Nutritionally, soybean is considered to be a rich source, with dry matter containing about 40% proteins and 20% fats (Shovan et al., 2008; Amrate, 2024). As a leguminous plant, it also enhances soil fertility through biological nitrogen fixation, contributing to sustainable agriculture. India stands fifth in global soybean production, after Brazil, the USA, Argentina, and China (Ray et al., 2022). Soybean occupies an area of 13.25 mha, yielding 13.06 million tonnes with a productivity of 985 kg ha^{-1} (Anonymous, 2024). In Telangana, there is an increase in soybean cultivation (Pallavi et al., 2015) covering 0.18 million hectares, with a production of 0.270 mt and productivity of 1483 kg ha^{-1}, ranking sixth in the country (Anonymous, 2024). Despite the crop's growing importance, diseases pose a major challenge to soybean productivity. The soybean seed-borne diseases have emerged as critical yield-limiting concerns as they impair seed quality, reduce germination and seedling vigour, leading to poor crop establishment (Sinclair, 1982; Chang et al., 2020; Dell'Olmo et al., 2023). Fungal deterioration of seeds typically results in seed discoloration, rot, and early seedling mortality (McGee et al., 1980; Hartman, 2015; Gebeyaw, 2020). Numerous studies have documented various fungal pathogens associated with soybean seeds, such as *Fusarium solani*, *F. oxysporum*, *F. pallidoroseum*, *F. semiticum*, *F. equiseti*, *Macrophomina phaseolina*, *Ascochyta sojicola*, *Cercospora kikuchii*, *Botrytis cinerea*, *Colletotrichum dematium*, *C. truncatum*, *C. gloeosporioides*, *Phomopsis sojae*, *Alternaria* spp., *Curvularia lunata*, *Aspergillus flavus*, *A. niger*, *Rhizopus stolonifer*, *Rhizoctonia* spp. and *Penicillium* spp. using agar plate and standard blotter methods (Agarwal et al., 2006; Ahammed et al., 2006, Shovan et al., 2008; Ramesh et al., 2013; Ahmed et al., 2016; Ibrahim, 2015; Escamilla et al., 2019; Soesanto et al., 2020; Kim et al., 2022; Khodifad and Sharma, 2022). Subsequently, most of these fungi were found to cause considerable reduction in seed germination and seedling growth (Shovan et al., 2008; Khodifad and Sharma, 2022). Economic losses of up to 59% have been documented due to wilt, 64% due to root rot, and 50% due to reduced pod development, and also infected seeds were reported to inhibit germination up to 40% in the field conditions (Hartman et al., 1999). Other research works also highlighted the impact of specific fungal species on seed quality. A wide range of *Fusarium* spp. associated with soybean seeds are pathogenic, and their detrimental effects are well-documented. It causes seed rot, seedling wilt, root rot, and pod decay and was found to be associated with reduced germination and poor seedling growth (Chang et al., 2020; Zhao et al., 2022). Lakshmeesha et al. (2013) found that *Macrophomina phaseolina* caused significant post-emergence damping-off in soybean seedlings, leading to crop losses of up to 50%, while Amrate et al. (2020) reported up to 86.5% mortality in the tested soybean genotypes due to charcoal rot. Similarly, *Colletotrichum* spp. causing anthracnose and *Diaporthe* spp. causing Phomopsis seed decay (PSD), particularly damage during pod-filling stages and can result in poor seed formation or seed quality, causing significant yield losses (Petrovic et al., 2021; Raghuwanshi et al., 2025). Considering the expansion of soybean cultivation and the prevalence of seed-borne fungal diseases, the present study focuses on the isolation and identification of seed-borne fungi and their effect on seed quality.

2. MATERIALS AND METHODS

2.1. Collection and categorization of soybean seed samples

Soybean seed samples were collected from major soybean-growing areas in the Adilabad, Nizamabad, Kamareddy and Jagtial districts of Telangana state during *kharif,* 2021 (June-September), directly from the farmers' fields. Among the samples, JS335 variety seeds were collected from all four districts. Additionally, Basara variety seed samples were collected from Adilabad district, and Karishma (hybrid) seed samples were collected from Jagtial district. The collected samples were categorized into four different grades: healthy, discoloured, shrivelled and broken seeds based on visual observation, and the 100-seed weight was also recorded for each sample (Ramesh et al., 2013).

2.2. Isolation of seed-borne mycoflora associated with soybean

The soybean seed samples collected from different locations were subjected to seed health tests using two methods: the standard blotter method and the agar plate method (Figure 1), following the guidelines of the ISTA-International Seed Testing Association (Anonymous, 1976).

2.2.1. Standard blotter method

In this method, three layers of sterilized blotter paper discs of 90 mm diameter were placed in each sterile Petri plate (90 mm diameter) and moistened with sterile distilled water. Soybean seeds were surface sterilized with 1% Sodium hypochlorite solution for one minute, followed by washing with three changes of sterile distilled water. Surface-sterilized seeds were placed on the moist blotter paper at equidistance (10 seeds $plate^{-1}$) and the plates were then incubated at 25±2°C for seven days in a BOD incubator. One hundred seeds were evaluated in each replication, with four replications maintained for each sample.

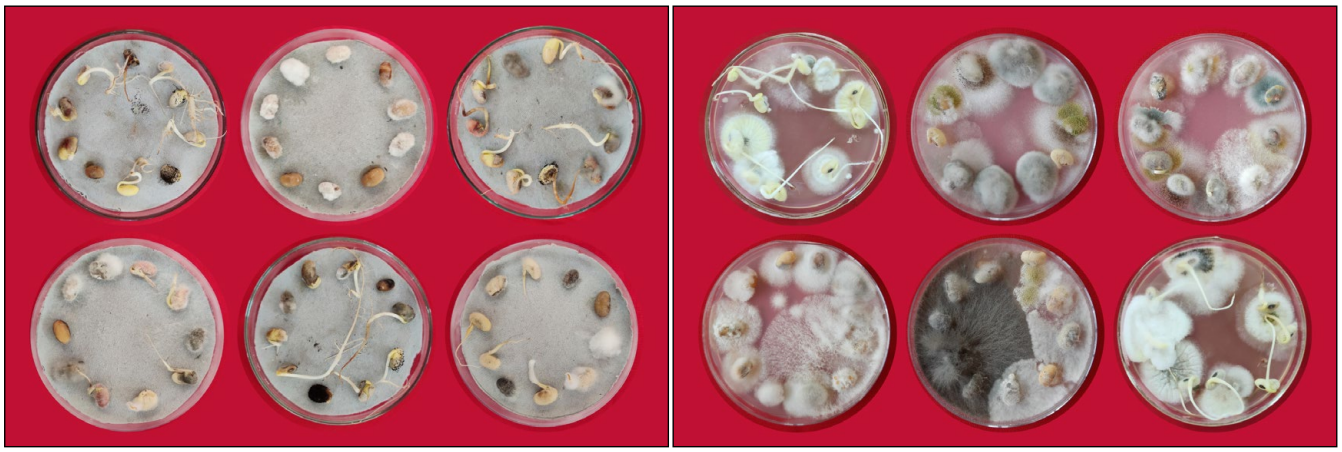

Figure 1: Isolation of soybean seed-borne mycoflora using the standard blotter method (left) and agar plate method (right)

2.2.2. Agar plate method

In this method, ten surface-sterilized seeds were placed equidistantly in each Petri plate containing PDA medium and incubated at 25±2°C for seven days in a BOD incubator. One hundred seeds were evaluated in each replication, with four replications maintained for each sample.

For both isolation methods, the plates were examined visually for seed infection till the eighth day of incubation. Mycelium and the asexual fruiting bodies/conidia of fungi were observed under a compound microscope. The total number of infected seeds was recorded in each plate, and the number of seeds infected by each specific fungus was also recorded separately.

To calculate the % seed infection and the frequency of occurrence of specific fungus, the following formulae were used (Kumar et al., 2020);

$$\text{Seed infection (\%)} = \frac{\text{Number of infected seeds}}{\text{Total number of seeds tested}} \times 100$$

$$\text{Frequency} = \frac{\text{Number of seeds on which a fungal species identified}}{\text{Total number of seeds tested}} \times 100$$

2.3. Studies on seed quality parameters of soybean samples

Soybean seed samples were subjected to seed quality tests using the rolled paper towel method. Four replications of 100 seeds from each sample were counted randomly and placed between two layers of moist germination paper towels, ensuring uniform spacing between the seeds. The paper towels were then rolled carefully and incubated vertically in a seed germinator at a temperature of 25±2°C and a relative humidity of 95±2% for one week.

2.3.1. Seed germination (%)

The germination % was recorded on the eighth day of incubation. In each replication, the total number of germinated seeds (healthy/normal seedlings) out of 100 seeds was counted, and the germination % was calculated using the following formula;

$$\text{Seed Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds kept for germination}} \times 100$$

2.3.2. Seed rot and seedling mortality

In each replication, the number of seeds that rotted and the number of seedlings showing mortality were recorded. The % of seed rot and seedling mortality was calculated using the following formulae;

$$\text{Seed rot (\%)} = \frac{\text{Number of seeds rotted}}{\text{Total number of seeds}} \times 100$$

$$\text{Seedling mortality (\%)} = \frac{\text{Number of seedlings showing mortality}}{\text{Total number of seeds}} \times 100$$

2.3.3. Seedling vigour index I (SVI-I)

The Seedling Vigour Index I (SVI-I) was calculated by multiplying the germination % with the mean seedling length (cm) of 10 randomly selected seedlings, as per the method suggested by Abdul-Baki and Anderson (1973). The formula is as follows;

Seedling vigour index I=Germination (%)×Mean seedling length (cm)

3. RESULTS AND DISCUSSION

3.1. Collection and categorization of soybean seed samples

A total of 62 soybean seed samples were collected from different locations in Telangana state. These samples included JS335 variety (predominant soybean variety grown in the state) from Adilabad, Nizamabad, Kamareddy and Jagtial districts. Additionally, Basara variety samples from Adilabad district and Karishma (hybrid) samples

from Jagtial district were also collected for this study. The collected seed samples were categorized into four different grades based on visual observation: healthy, discoloured, shrivelled, and broken (Figure 2), and the data are presented in Table 1. On average, 66.00% healthy, 18.33% discoloured, 12.00% shrivelled, and 3.67% broken seeds were found in the collected soybeans.

Basara variety samples from Adilabad showed the highest % (82.00%) of healthy seeds, followed by Karishma (79.00%) from Jagtial district. The lowest % (53.00%) of healthy seeds was recorded in JS335 samples from Nizamabad district, which also showed the highest % (30.00%) of discoloured seeds.

The 100-seed weight (in grams) was also recorded for all the collected seed samples. Among all the seed samples, the Basara variety from Adilabad showed the lowest % (3.00%) of shrivelled seeds and recorded the highest 100-seed weight (12.5 g). On the other hand, JS335 seeds from Jagtial district had the highest % (20.00%) of shrivelled seeds and the lowest 100-seed weight (9.8 g). The overall proportion of broken seeds ranged from 2%-6%, with an average of 3.67%, which was relatively low compared to the other seed categories.

Figure 2: Categorization of soybean seed samples into different grades based on visual observation

Similar variations in the proportion of discoloured, shrivelled, broken, and other seed categories, assessed through visual inspection and dry seed examination for fungal infections, have been reported in soybean by Agarwal et al. (2006), Ramesh et al. (2013), and Ahmed et al. (2016), and in maize by Sadia and Shamsi (2020). Most fungi were also found to cause discoloration of the seed coat, which is a major factor that impedes seed quality (Gebeyaw, 2020). This variation in seed categorization often leads to a decrease in seed weight, with an increase in discoloured, shrivelled, and broken seeds. Similar findings were reported by Krishnamurthy et al. (2003) in cowpea, horse gram, black gram, and green gram. Ray et al. (2022) also reported that seed size exhibited a significant positive correlation with 100-seed weight and seed yield plant^{-1}.

3.2. Isolation of seed-borne mycoflora associated with soybean

Isolation of seed-borne mycoflora from the collected soybean seed samples was done by standard blotter and agar plate methods, as per the procedures followed by ISTA (Anonymous, 1976). Different fungi observed on seeds were isolated into pure cultures and identified using published fungal identification keys.

A total of eight fungal species belonging to six genera

Table 1: Categorization of soybean seed samples collected from soybean-growing areas of Telangana based on visual observation

District	Variety/Hybrid	100-seed weight (g)	Grades of seed (%)			
			Healthy	Discoloured	Shrivelled	Broken
Adilabad	JS335	10.80	56	26	16	2
	Basara	12.50	82	9	3	6
Nizamabad	JS335	11.10	53	30	14	3
Kamareddy	JS335	11.50	65	19	11	5
Jagtial	JS335	9.80	61	15	20	4
	Karishma	12.10	79	11	8	2
Mean		11.30	66.00	18.33	12.00	3.67
SEm±		0.09	0.82	0.51	0.33	0.10
CD (p≤0.05)		0.27	2.54	1.59	1.04	0.32

were recorded using both the standard blotter and agar plate methods. These fungi included *Fusarium oxysporum*, *Fusarium semitectum*, *Macrophomina phaseolina*, *Colletotrichum truncatum*, *Curvularia* spp., *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* spp. as presented in Figure 3. These fungi were found to be seed-borne in soybean, as reported by several researchers using both methods of detection (Wan, 1980; Ahammed et al., 2006; Shovan et al., 2008; Rao et al., 2015; Soesanto et al., 2020; Khodifad and Sharma, 2022). The total seed infection rate and the % incidence of fungi in each method were recorded.

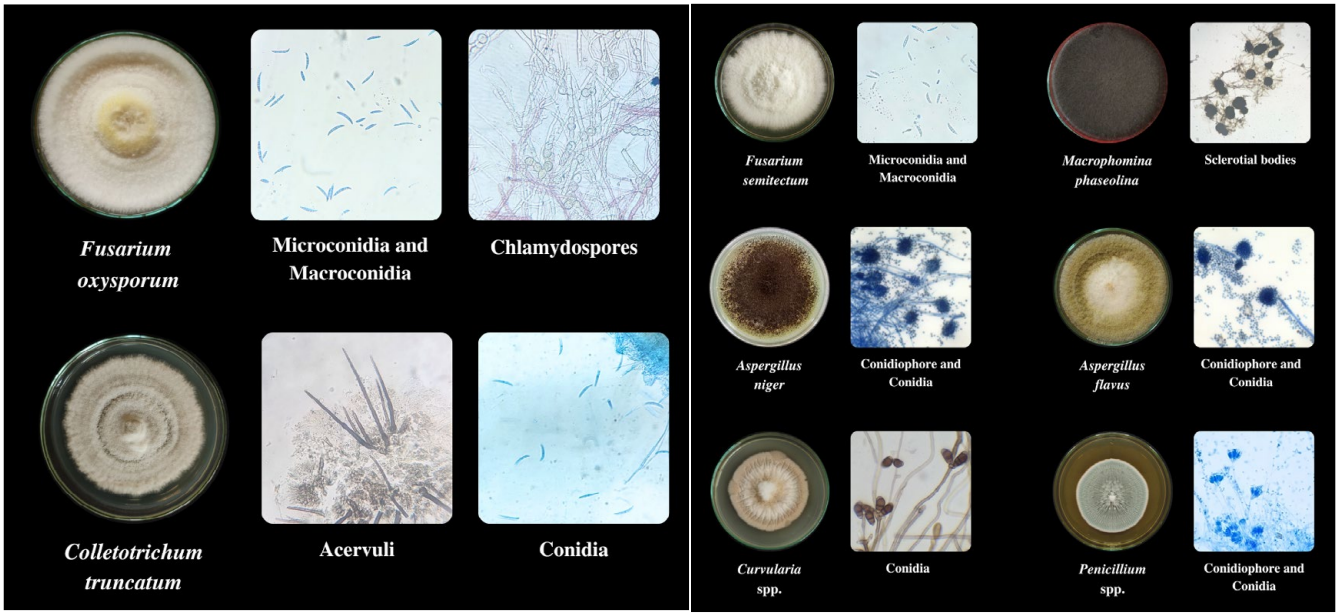

Figure 3: Pure cultures and photomicrographs (40X and 10X) of seed-borne mycoflora isolated from soybean seeds

3.2.1. Standard blotter method

The data pertaining to total seed infection % and the frequency of occurrence of individual seed mycoflora in soybean, analysed using the standard blotter method, are presented in Table 2. The results indicate a significant difference in total seed infection, ranging from 21.21%–49.02%. Among the samples collected, the Basara variety from Adilabad district recorded the lowest total seed infection (21.21%), followed by hybrid Karishma (26.50%) from Jagtial district. In contrast, seed samples of JS335 variety from Nizamabad district recorded the highest total seed infection (49.02%), followed by Adilabad (43.95%), Kamareddy (38.56%) and Jagtial (36.26%) districts. Similar seed infection patterns observed in different districts of Telangana State were previously reported by Rao et al. (2015). Pallavi et al. (2015) also reported that among different genotypes studied, the Basara variety recorded the highest grain yield during the *kharif* season in Northern

Table 2: Total seed infection and frequency of mycoflora recorded in collected soybean seed samples by standard blotter method

District	Variety/ Hybrid	Total seed infection (%)	Frequency of isolated mycoflora (%)			
			Fusarium oxysporum	*Fusarium semitectum*	*Macrophomina phaseolina*	*Colletotrichum truncatum*
Adilabad	JS335	43.95	15.94 (23.52)	6.88 (15.19)	12.25 (20.48)	1.50 (7.02)
	Basara	21.21	8.19 (16.62)	2.81 (9.64)	5.88 (14.02)	1.19 (6.23)
Nizamabad	JS335	49.02	18.50 (25.46)	8.13 (16.55)	5.88 (14.02)	0.94 (5.54)
Kamareddy	JS335	38.56	14.63 (22.47)	0.00 (0.00)	10.50 (18.90)	1.19 (6.23)
Jagtial	JS335	36.26	11.69 (19.98)	5.88 (14.02)	9.94 (18.37)	0.00 (0.00)
	Karishma	26.50	9.38 (17.82)	6.00 (14.17)	6.75 (15.05)	0.00 (0.00)
Mean		35.92	13.05	4.95	9.51	0.80
Factors	District and Variety (A)		Seed-borne mycoflora (B)		Factor (A x B)	
SEm±	0.04		0.05		0.12	
CD ($p \leq 0.05$)	0.12		0.14		0.34	

Table 2: Continue...

District	Variety/ Hybrid	Total seed infection (%)	Frequency of isolated mycoflora (%)			
			Curvularia spp.	*Aspergillus niger*	*Aspergillus flavus*	*Penicillium spp.*
Adilabad	JS335	43.95	0.63 (4.51)	2.44 (8.97)	3.56 (10.87)	0.75 (4.93)
	Basara	21.21	1.00 (5.71)	0.88 (5.35)	1.13 (6.08)	0.13 (1.43)
Nizamabad	JS335	49.02	1.75 (7.59)	2.63 (9.31)	4.19 (11.80)	1.13 (6.06)
Kamareddy	JS335	38.56	1.81 (7.72)	3.00 (9.97)	5.13 (13.07)	2.31 (8.73)
Jagtial	JS335	36.26	1.00 (5.74)	2.69 (9.41)	3.25 (10.37)	1.81 (7.72)
	Karishma	26.50	0.81 (5.12)	1.19 (6.23)	1.81 (7.72)	0.56 (4.18)
Mean		35.92	1.17	2.14	3.18	1.12
Factors	District and Variety (A)		Seed-borne mycoflora (B)		Factor (A x B)	
CD ($p \leq 0.05$)	0.04		0.05		0.12	
C.D.	0.12		0.14		0.34	

Figures in the parentheses are angular transformed values

Telangana Zone and is highly suitable for cultivation.

The frequency of occurrence of isolated fungi varied across different locations. Irrespective of location, the mean frequency of *F. oxysporum* (13.05%), followed by *M. phaseolina* (9.51%) was found to be highest among all the fungi, and the least mean frequency of occurrence was observed with *C. truncatum* (0.80%).

The highest frequencies of *F. oxysporum* (18.50%) and *F. semitectum* (8.13%) were recorded in JS335 from Nizamabad, followed by Adilabad (15.94% and 6.88%, respectively), and the lowest (8.19% and 2.81%, respectively) in the Basara variety of Adilabad. Notably, *F. semitectum* was completely absent in samples from Kamareddy.

The pathogen *M. phaseolina* recorded the highest frequency (12.25%) in JS335 samples from Adilabad, followed by Nizamabad (11.75%), Kamareddy (10.50%), and Jagtial (9.94%), while the lowest frequency (5.88%) was recorded in the Basara variety from Adilabad, followed by Karishma from Jagtial (6.75%). Similarly, the frequency of occurrence of *C. truncatum* was recorded highest (1.50%) in JS335 samples from Adilabad, followed by Basara from Adilabad and JS335 from Kamareddy districts, which recorded similar frequencies of 1.19%, while the least frequency (0.94%) was observed in JS335 samples from Nizamabad. Interestingly, *C. truncatum* was completely absent in seed samples from Jagtial district. *A. flavus*, *A. niger*, *Curvularia* spp., and *Penicillium* spp. were detected in all the collected seed samples. The highest frequency of occurrence for each of these fungi, viz., *A. flavus* (5.13%), *A. niger* (3.00%), *Curvularia* spp. (1.81%) and *Penicillium* spp. (2.31%) was recorded in JS335 samples from Kamareddy district.

From the above results, it was observed that the occurrence of fungi varied across locations. For instance, *F. semitectum* was completely absent in JS335 seed samples from Kamareddy, while none of the seed samples from Jagtial district recovered *Colletotrichum* spp. Similar variations in the frequency of occurrence of seed-borne fungi have been reported in earlier studies (Shovan et al., 2008; Rao et al., 2015).

3.2.2. Agar plate method

The data of the total seed infection % and the frequency of occurrence of individual seed mycoflora in soybean, analysed through the agar plate method, are presented in Table 3. The results indicate a significant difference in total seed infection, ranging from 19.76%-44.64%. Among the samples collected, Basara variety seeds from Adilabad district recorded the lowest total seed infection (19.76%), followed by Karishma (23.07%) from Jagtial district. JS335 seed samples from Nizamabad district recorded the highest total seed infection (44.64%), followed by JS335 seed samples from Adilabad (40.39%), Kamareddy (35.50%), and Jagtial (33.15%) districts, respectively.

Across the varieties, the Basara variety from Adilabad recorded the lowest total seed infection (19.76%), while JS335 from Nizamabad recorded the highest total seed infection (44.64%). Karishma seed samples from Jagtial district had 23.07% of total seed infection, which was comparatively lower than the seed infection % recorded for JS335 seeds from all districts.

The frequency of occurrence of fungi varied across locations. Irrespective of location, the mean frequency of *F. oxysporum* (10.03%), followed by *M. phaseolina* (8.70%), was found to be highest among all fungi observed, while the least mean frequency was observed for *C. truncatum* (0.50%), similar to that of the standard blotter method. The highest frequency of *F. oxysporum* (13.13%), *F. semitectum* (6.63%) and *M. phaseolina* (12.06%) were recorded in JS335 samples from Nizamabad, followed by Adilabad (12.75%, 5.25% and

Table 3: Total seed infection and frequency of mycoflora recorded in collected soybean seed samples by agar plate method

District	Variety/ Hybrid	Total seed infection (%)	Frequency of isolated mycoflora (%)			
			Fusarium oxysporum	*Fusarium semitectum*	*Macrophomina phaseolina*	*Colletotrichum truncatum*
Adilabad	JS335	40.39	12.75 (20.91)	5.25 (13.24)	10.63 (19.02)	0.50 (3.98)
	Basara	19.76	6.44 (14.69)	2.50 (9.09)	5.50 (13.56)	1.25 (6.37)
Nizamabad	JS335	44.64	13.13 (21.23)	6.63 (14.91)	12.06 (20.31)	0.63 (4.41)
Kamareddy	JS335	35.50	9.75 (18.19)	0.00 (0.00)	8.69 (17.13)	0.63 (4.51)
Jagtial	JS335	33.15	9.88 (18.31)	4.19 (11.80)	9.38 (17.82)	0.00 (0.00)
	Karishma	23.07	8.25 (16.68)	4.63 (12.41)	5.94 (14.10)	0.00 (0.00)
Mean		32.75	10.03	3.87	8.70	0.50
Factors	District and Variety (A)		Seed-borne mycoflora (B)		Factor (A x B)	
SEm±	0.05		0.06		0.14	
CD ($p \leq 0.05$)	0.13		0.15		0.38	

District	Variety/ Hybrid	Total seed infection (%)	Frequency of isolated mycoflora (%)			
			Curvularia spp.	*Aspergillus niger*	*Aspergillus flavus*	*Penicillium* spp.
Adilabad	JS335	40.39	1.25 (6.40)	4.50 (12.24)	2.88 (9.75)	2.63 (9.31)
	Basara	19.76	1.00 (5.71)	1.44 (6.87)	1.00 (5.66)	0.63 (4.51)
Nizamabad	JS335	44.64	1.00 (5.64)	5.13 (13.07)	3.81 (11.25)	2.25 (8.61)
Kamareddy	JS335	35.50	1.25 (6.37)	6.69 (14.98)	4.31 (11.98)	4.19 (11.79)
Jagtial	JS335	33.15	0.63 (4.51)	3.88 (11.35)	3.19 (10.28)	2.00 (8.12)
	Karishma	23.07	0.50 (3.98)	1.69 (7.45)	1.00 (5.71)	1.06 (5.91)
Mean		32.75	0.94	3.89	2.70	2.13
Factors	District and Variety (A)		Seed-borne mycoflora (B)		Factor (A x B)	
SEm±	0.05		0.06		0.14	
CD ($p \leq 0.05$)	0.13		0.15		0.38	

Figures in the parentheses are angular transformed values

10.63%, respectively), with the lowest (6.44%, 2.50% and 5.50%, respectively) in the Basara variety from Adilabad. Notably, *F. semitectum* was completely absent in seed samples from Kamareddy, as observed using the standard blotter method.

The frequency of occurrence of *C. truncatum* was highest (1.25%) in Basara samples from Adilabad, followed by JS335 from Nizamabad and Kamareddy (0.63%), and the lowest frequency (0.50%) was observed in JS335 from Adilabad. *C. truncatum* was completely absent in seed samples from Jagtial district. Both *A. niger* and *A. flavus* were detected in all collected seed samples, with the highest frequencies recorded in JS335 from Kamareddy (6.69% and 4.31%, respectively). *Curvularia* spp. and *Penicillium* spp. were detected in all seed samples, with *Penicillium* spp. showing the highest frequency (4.19%) in JS335 from Kamareddy, while *Curvularia* spp. (1.25%) in JS335 from both Adilabad and Kamareddy.

Similar variations in the occurrence of fungi concerning location and variety were previously reported in soybean. Rao et al. (2015) collected soybean seed samples (cv. JS-335) from Adilabad and Nizamabad districts of Telangana and detected nine seed-borne fungal species. The incidence of seed mycoflora ranged from 30–49.2% and 23.6–45.0% by blotter method, and 15.4–26.4% by agar plate method, respectively. Among the fungi, *M. phaseolina* was predominant, while *Cladosporium* sp. was least frequent. Similarly, Escamilla et al. (2019) isolated seven fungal species from soybean, with *Fusarium* spp. (30.77%) being the most common fungi across locations tested.

3.2.3. Comparison between standard blotter and agar plate methods

Across both blotter and agar plate methods, for the detection of seed-borne mycoflora in soybean, *F. oxysporum* was the most predominant fungus, followed by *M. phaseolina*. The predominance of *F. oxysporum* and *M. phaseolina* in

soybean seed lots was also reported by Ramesh et al. (2013). Location-specific variation was observed in the frequency of recovered fungi, suggesting that environmental conditions during seed development influence fungal occurrence. Nevertheless, *F. oxysporum*, *M. phaseolina*, *Curvularia* spp., *A. niger*, *A. flavus*, and *Penicillium* spp. were recovered in all four districts, though at varying degrees of seed infection. Similar findings on variations in seed infection by location were reported by Ramesh et al. (2013).

The total seed infection % was higher in the standard blotter method as compared to the agar plate method. The efficacy of both methods varied depending on the nature and type of fungi. Both the methods were effective in recovering a large number of mycoflora, but on comparison, the standard blotter method was found to be superior for isolating most seed-borne fungi associated with soybean, including *F. oxysporum*, *F. semitectum*, *M. phaseolina*, *C. truncatum*, *Curvularia* spp. and *A. flavus*. On the other hand, the agar plate method was found to be more effective in recovering *A. niger* and *Penicillium* spp. over the standard blotter method, as can be observed from Figure 4. Similar observations were

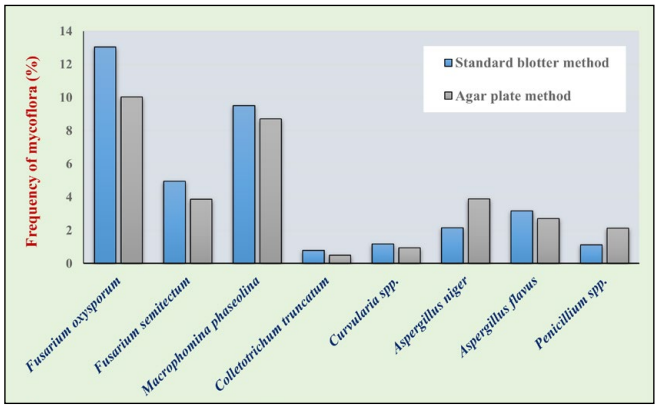

Figure 4: Comparative frequency of seed-borne mycoflora of soybean isolated using standard blotter and agar plate methods

made by Ahmed et al. (2016), who found the agar plate method to be more efficient in isolating *Aspergillus* spp. and *Penicillium* spp., while the blotter method was more effective for *Fusarium* spp. The superiority of the standard blotter method over the agar plate method in isolating seed-borne mycoflora in soybean has been reported previously by Neergaard (1977), Wan (1980), Arya et al. (2004), Ramesh et al. (2013), Rao et al. (2015), and Soesanto et al. (2020).

3.3. Studies on seed quality parameters of soybean samples

The soybean seed samples collected were also evaluated for seed rots, seedling infections and other seed quality parameters using the rolled paper towel method (Figure 5), as per the procedures of ISTA (Anonymous, 1976), and the data of the same are presented in Table 4. The results

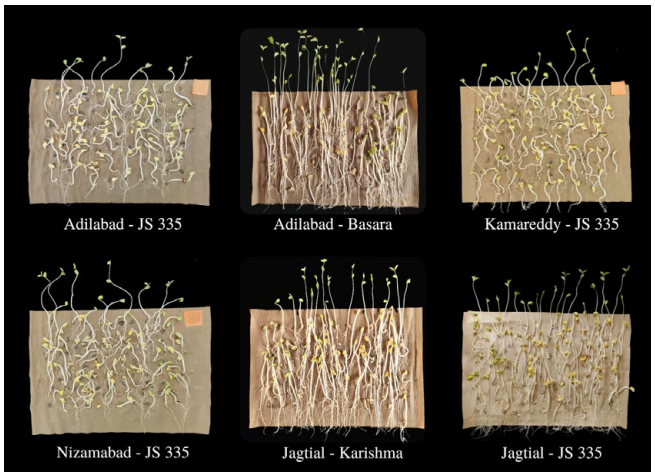

Figure 5: Evaluation of seed quality parameters of soybean seed samples using the rolled paper towel method

show significant variation in seed germination (64.63%–85.05%), seed rot (6.25%–24.18%), seedling mortality (8.70%–12.10%), and seedling vigour index-I (1496–3049).

Among all the seed samples, the Basara variety from Adilabad recorded the significantly highest seed germination

Table 4: Evaluation of seed quality parameters of soybean seed samples using the rolled paper towel method

District	Variety/Hybrid	Germination (%)	Seed rot (%)	Seedling mortality (%)	Mean seedling length (cm)	Vigour index-1
Adilabad	JS335	69.38 (56.38)	18.53 (25.48)	12.10 (20.34)	23.15	1606
	Basara	85.05 (67.23)	6.25 (14.47)	8.70 (17.15)	35.85	3049
Nizamabad	JS335	64.63 (53.48)	24.18 (29.44)	11.20 (19.54)	23.15	1496
Kamareddy	JS335	73.13 (58.75)	16.38 (23.86)	10.50 (18.90)	26.15	1912
Jagtial	JS335	74.83 (59.86)	15.80 (23.41)	9.38 (17.82)	25.85	1934
	Karishma	79.03 (62.72)	10.63 (19.01)	10.35 (18.76)	29.08	2298
CD ($p \leq 0.05$)		0.44	0.48	0.66	0.84	64.04
SEm±		0.15	0.16	0.22	0.28	21.39
C.V.		0.49	1.40	2.36	2.05	2.09

Figures in the parentheses are angular transformed values

(85.05%), followed by Karishma (79.03%) from Jagtial district. This higher germination could be attributed to the lowest seed infection % in these respective samples, as observed in Tables 2 and 3, and was likely influenced by the higher % of healthy seeds, as shown in Table 1. On the other hand, the lowest seed germination (64.63%) was recorded in JS335 samples from Nizamabad district, which might be due to the highest recovery of seed-borne fungi recorded in these samples and the highest % of deformed seeds. This finding indicates a positive correlation between seed deformities, 100-seed weight, and seed infection %, as shown in Tables 1, 2 and 3. These results are consistent with the findings of Shovan et al. (2008); Ramesh et al. (2013); Chang et al. (2020) and Ray et al. (2022).

The lowest seed rot (6.25%) and seedling mortality (8.70%) were recorded in the Basara variety from Adilabad. Conversely, the highest seed rot (24.18%) was recorded in JS335 samples from Nizamabad, while the highest seedling mortality (12.10%) was recorded in JS335 samples from Adilabad, followed by Nizamabad (11.20%) and other samples, as shown in Table 4.

The highest seedling vigour index-I (3049) was recorded in the Basara variety from Adilabad, followed by Karishma from Jagtial (2298), JS335 from Jagtial (1934), Kamareddy (1912), and Adilabad (1606) districts, respectively. The lowest seedling vigour index-I (1496) was recorded in JS335 samples from Nizamabad district.

These results are in agreement with findings of Meena Kumari et al. (2002); Ahammed et al. (2006); Chang et al. (2020); Soesanto et al. (2020); Khodifad and Sharma (2022) and Zhao et al. (2022), who reported that pathogenic seed-borne fungi can be associated with reduced germination, seedling growth and other seed quality parameters in soybean.

4. CONCLUSION

Mycoflora associated with soybean seeds were recorded using standard blotter and agar plate methods. Both methods were effective in recovering diverse mycoflora, though their efficiency varied with fungal type. Results indicated a positive correlation between seed deformities and infection percentage, while reduction in seed quality parameters including germination was also observed due to pathogenic fungi.

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