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Impact of Isolated Seed Mycoflora on Seedling Vigour Index of Yardlong Bean Seeds

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Abstract

The present investigation was conducted during the year 2022–2023 at the laboratory of Department of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University, Navsari to isolate seed mycoflora associated with yardlong bean seeds and to study their impact on seedling vigour index. Seed mycoflora was isolated by three different methods viz., agar plate method, standard blotter paper method and deep-freezing method. Pathogenicity tests were conducted under *in-vitro* and *in-vivo* conditions and dominant mycoflora were selected to further check their impact on seed health status of yardlong bean seeds. Total ten fungi were isolated viz*., Aspergillus niger*, *A. flavus*, *Penicillium* sp., *Rhizopus* sp., *Fusarium* sp., *Colletotrichum* sp., *Curvularia* sp., *Alternaria* sp., *Botryodiplodia* sp. and *Sclerotium* sp. *Botryodiplodia* sp., *Curvularia* sp., *Colletotrichum* sp., *Fusarium* sp. and *Sclerotium* sp., being dominant, were selected and further used to check their impact on seed germination, shoot length, root length and seedling vigour index of yardlong bean by using paper towel method. *Fusarium* sp. and *Sclerotium* sp. showed a considerable decrease in seed germination, root length, shoot length and seedling vigour index of yardlong bean over control.

Keywords: Seed mycoflora, yardlong bean, seedling vigour index

1. Introduction

In the field of botany, one of the three largest categories of flowering plants is the *Fabaceae* family, formerly referred to as *Leguminosae*, which encompasses beans (Jin et al., 2019). The family is classified into three sub-families, *Papilionoideae*, *Phaseoleae* and *Phaseolinae* accounting for around two-thirds of all species according to the modern nomenclature (Gepts, 2001). The Vigna genus includes different crops such as cowpea, mung bean, urd bean, adzuki bean, bambara groundnut, mat bean and rice bean which have considerable economic importance in many developing countries (Fery, 2002).

Yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.) belongs to the *Leguminosae* family which is mainly grown in tropical areas (Devan et al., 2021). It originated in Southeast Asia and southern China, where it has been cultivated since ancient times. It is generally known as vegetable cowpea, chinese long bean, string bean, snake bean, snap pea, pea bean, long-podded cowpea, bodi and bora. It is a vigorous climbing annual vine and grown primarily for its strikingly long (35 to 75 cm) immature pods which has uses very similar to that of the green bean. The varieties of yard long beans are usually distinguished by the different colors of their mature seeds (Rachie, 1985; Dechjirarattanasiri and Inthasan, 2024). The mature pods of yardlong bean cultivars have protein approximately 3 g, carbohydrate 8.35 g and 0.4 g fat. Also, it contains lot of vitamin A, B and C, folate, thiamin, riboflavin, iron, phosphorus, potassium, magnesium and manganese (Carbonaro and Nucara, 2022). Generally, there are two varieties one with red or purple pods and the other one has green pods. In India, the green variety is more preferred (Anonymous, 2023). Yarddlong bean is widely grown in Southeast Asia, South China, Indonesia, Philippines, Taiwan and Thailand (Rachie, 1985). Now-a-days, it is being cultivated in several states of India viz., Andhra Pradesh, Kerala, Karnataka and Maharashtra having over an area of 18,560 to 20,160 ha annually (Ano and Ubochi, 2008). In Gujarat state, yardlong bean is recently introduced crop and gaining its popularity among farming community as cultivated in isolated pockets and mostly in kitchen gardens.

Seeds play a crucial role in crop production but are often colonized by seed-borne fungi, many of which are pathogenic and pose a significant threat to seedling establishment. These fungi can cause seed abortion, shrinkage, reduced size, rot, sclerotization, stromatization, necrosis, discoloration, and loss of germinability, along with physiological alterations. During harvest, transport, processing, and storage, fungal contamination leads to detrimental changes, making seeds unfit for sowing and consumption. Additionally, seeds act as passive carriers of pathogens, facilitating disease transmission under favourable environmental conditions (Amza, 2018; Chaudhari et al., 2017; Mehrotra and Aggarwal, 2003).

Seed-borne pathogens, particularly fungal mycoflora, can significantly impact crop health and productivity. These fungi not only cause various diseases but also reduce seed viability, storage quality, germination rate, seedling vigour, nutritional value, and overall yield. Successful crop establishment depends on strong germination and seedling growth, both of which can be severely hindered by seed-borne fungi, whether acting alone or in combination (Rathod et al., 2021).

Seed mycoflora of asparagus vigna (*Vigna unguiculata* (L.) Walp.) viz., *Fusarium* spp., *Mucor mucedo* Sowerby, *Penicillium* spp., *Rhizopus* spp., *Aspergillus niger* Tiegh., *Alternaria* spp., *Botrytis cinerea* Pers. and *Sclerotinia sclerotiorum* (Lib.) de Bary reported to contaminate the seeds (Fotev and Kazakova, 2020). Seed mycoflora which are described earlier can deteriorate the quality of yardlong bean seeds which, directly effects the yardlong bean cultivation. Therefore, the present investigation was under taken to find out the mycoflora associated with the yardlong bean seeds and its effect on seed and seedling quality.

2. Materials and Methods

2.1. Experimental location

The present investigation was conducted at Department of Plant Pathology, N. M. College of Agriculture (NMCA), Navsari Agricultural University (NAU), Navsari, Gujarat (396 450), India during the year 2022-2023.

2.2. Source of experimental material

The seed sample was collected from the Regional Horticulture Research Station (RHRS), Navsari Agricultural University (NAU), Navsari, Gujarat (396 450), India. Collected seed sample was stored in brown paper bag at laboratory of Department of Plant Pathology, NMCA, NAU for further investigation.

2.3. Isolation and identification of fungi

2.3.1. Isolation of fungi

For isolation of seed mycoflora of yardlong bean, three methods were used viz., agar plate method, standard blotter paper method and deep-freezing method.

In agar plate method, five seeds were placed in each Petri plate and incubated at 25±2°C in BOD incubator. After 48 hrs the incubated plates were observed under the stereoscopic microscope. The observations were continued for 10 days to check the presence of seed-borne mycoflora. The observations were taken to calculate the percentage of seed borne fungi.

In standard blotter paper method, ten seeds per plate were placed at equidistance in a circle in plates containing moistened paper discs. The plates were incubated at 25±2°C for seven days under alternate cycles of 12 hrs light and 12 hrs darkness for 7 days in BOD incubator. The plates were examined under stereo-binocular microscope on 8th day for presence of seed-borne mycoflora.

Deep freezing method was almost same as the blotter paper method except the Petri plates containing yardlong bean seeds were first incubated at 25±2°C for one day then after it transferred to deep freezer at 20°C for 24 hrs. Deep freezed Petri plates were again incubated at 25±2°C for 5 days. After one week, the Petri plates were observed for presence of various fungi. The observation was continued for ten days. The observations were taken to calculate the percentage of seed borne fungi.

2.3.2. Identification of fungi

Various seed borne fungi were observed on yardlong bean seeds after incubation. These fungi were separately cultured on PDA Petri plates. Each fungal growth was critically observed under microscope for cultural and morphological characters. Finally, observed fungal characteristics were compared with the characteristics described in various published literature and identification was done by referring authentic relevant literature (Neergaard, 1977; Kanapathipillai, 1982; Ahmed and Reddy, 1993; Thakur et al., 2010; Fotev and Kazakova, 2020). The isolated cultures were maintained on PDA slants by sub culturing and stored at 5°C for further study.

2.4. Pathogenicity test under in vitro and in vivo conditions

Under *in vitro* condition, the surface sterilized healthy seeds of yardlong bean were inoculated with 10 days old culture of each test fungus. For one repetition, 100 seeds for each fungus as four rolled paper towels with 25 seeds per paper towel were placed, whereas un-inoculated seeds served as control. Total four repetitions were carried out.

Under *in vivo* condition, the surface sterilized apparently healthy seeds of yardlong bean were inoculated with 10 days old culture of each test fungus and 10 seeds per pot were sown on previously prepared plastic pots containing sterilized soil. The un-inoculated seeds served as control. The observations on germination were recorded 8th and seedling mortality on 15th DAS. Pre-emergence and post-emergence mortality were calculated by following formula (Singh et al., 2022):

Pre-emergence mortality (%)=(Number of ungerminated seeds pot^{-1}/Number of sown seeds pot^{-1})×100

Post-emergence mortality (%)=(Number of died seedling/ Number of survived seedling)×100

2.5. Impact of mycoflora on seed health status

Impact of seed borne fungi on seed health status was studied in respect of seed germination ability and seedling vigour from artificially inoculated seeds with fungi isolated from naturally infected yardlong bean seeds. The experiment included six treatments in a completely randomized design with four repetitions. Healthy yardlong bean seeds artificially inoculated with each of the five fungal species based on their pathogenic nature viz., *Botryodiplodia* sp., *Curvularia* sp., *Colletotrichum* sp., *Fusarium* sp. and *Sclerotium* sp. separately. For artificial inoculation, seeds were moistened with sterilized distilled water and thoroughly mixed with fungal cultures that had been grown for 10 days, containing a concentration of 1×10^6 colony-forming units per millilitre. First paper towel was wetted and 25 seeds were placed on it followed by placing of second wetted paper towel on the first sheet and both the towels were rolled. Rolled papers were incubated in seed germinator at 25°C for 7 days. At the end of incubation period, rolled paper towels were carefully opened. Germinated and non-germinated seeds were counted from each of the treatments. The emergence of seedling from the seeds was considered as successful germination. After the end of incubation period, these seeds were used for study of seed germination and seedling vigour index. Uninoculated seeds served as control for comparison. Seedling vigour index (SVI) was determined based on seed germination as well as shoot and root length of seedlings.

Vigour index was calculated by using following formula (Abdul-Baki and Anderson, 1973):

Seed germination (%)=(Number of seed germinated/Total number of seeds)×100

The formula is:

Seedling vigour index (SVI)=% seed germination×(mean of root length+mean of shoot length in cm)

2.6. Statistical analysis

The data collected under study were subjected to the statistical analysis for proper interpretation. The standard method of analysis of variance technique appropriate to the Completely Randomized Design (CRD) as described by (Panse and Sukhatme, 1967) was used. The data were analyzed with the technical help received from Department of Agril. Statistics, N. M. College of Agriculture, NAU, Navsari. The treatment differences were tested by employing 'F' test at five per cent level of significance on the basis of null hypothesis. The appropriate standard error of mean (SEm) was calculated and the critical difference (CD) at five per cent level of probability were worked out to compare the two treatment means. The coefficient of variation percentage (CV%) was also worked out for all the cases to understand the variability present in the experimental unit.

3. Results and Discussion

3.1. Prevalence of naturally occurring fungi on yardlong bean seeds

Seed-borne mycoflora from composite sample of yardlong bean seeds were isolated by agar plate method, standard blotter paper method and deep-freezing method. Both the surface sterilized and non-surface sterilized seeds revealed the association of ten different predominant fungi. These fungi were initially designated as isolates number 1 to 10 (Table 1). The different isolates viz., *Aspergillus niger*, *A. flavus*, *Penicillium* sp., *Rhizopus* sp., *Fusarium* sp., *Colletotrichum*

Table 1: Percentage of naturally occurring seed mycoflora on yardlong bean through different isolation methods

Sl. No.	Isolates	Detection methods					
		Agar plate method* (%)		Standard blotter paper method* (%)		Deep-freezing method* (%)	
		SS	US	SS	US	SS	US
		Pathogens associated					
1.	*Aspergillus niger*	22.75	29.50	21.00	25.75	07.50	10.50
2.	*Aspergillus flavus*	23.75	26.00	22.25	26.75	08.00	11.00
3.	*Penicillium* sp.	15.25	21.00	11.50	21.75	05.50	08.00
4.	*Rhizopus* sp.	07.25	24.50	07.50	12.00	00.75	02.25
5.	*Fusarium* sp.	16.25	07.75	10.25	11.50	09.00	11.75
6.	*Colletotrichum* sp.	14.00	10.75	08.00	09.50	07.25	09.00
7.	*Curvularia* sp.	06.75	09.00	01.75	02.00	00.50	01.25
8.	*Alternaria* sp.	03.50	05.50	01.25	02.50	00.75	01.00
9.	*Botryodiplodia* sp.	03.75	11.75	00.25	00.75	00.25	00.00
10.	*Sclerotium* sp.	03.00	07.50	00.00	00.50	00.75	04.50

*Average of four repetitions; SS: Surface sterilized; US: Un-sterilized

sp., *Curvularia* sp., *Alternaria* sp., *Botryodiplodia* sp. and *Sclerotium* sp. were found to be associated with the seeds of yardlong bean.

The results are more or less similar with Jyoshna and Saxena (2021) who reported that most common fungal genera associated with cowpea seed were *Aspergillus* spp., *Fusarium* spp., *Pencillium* spp. and *Rhizopus* spp. using blotter paper method and agar plate method. Arfin et al. (2025) used dry seed inspection and blotter method for the detection of seed borne pathogen associated with yardlong beans collected from three shops located in Siddik Bazar, Dhaka. The seeds for each crop were sourced from Mithila Seed Enterprise, Alo Bij Vander, and Bismillah Seed Store. They reported that incidence of *Aspergillus flavus* ranged from 8.0% to 9.0%. The highest incidence (9.0%) was observed in seeds collected from Alo Bij Vander, which was similar to the incidence in seeds from Mithila Seed Enterprise. The lowest incidence (8.5%) was observed in seeds collected from Bismillah Seed Store. Statistically, no significant differences were observed for seed-borne infections of *Rhizopus* spp., *Aspergillus niger*, *Fusarium* sp., and *Chaetomium* spp. among the samples.

3.2. Pathogenicity test under in vitro and in vivo condition

3.2.1. Pathogenicity test under in vitro

The data presented in table 2 revealed that inoculation of all fungi isolated from seeds of yardlong bean has reduced germination and caused pre-emergence and post-emergence mortality as compared to un-inoculated seeds. Per cent pre-emergence mortality was found higher in the seeds inoculated with the *Botryodiplodia* sp. (74.50%) as compared to the rest of mycoflora fungi. Next higher pre-emergence mortality was recorded in *Colletotrichum* sp. (74.25%) followed by *Curvularia* sp. (70.00%), *Sclerotium* sp. (69.00%), *Rhizopus* sp. (68.00%), *Fusarium* sp. (65.50%), *Aspergillus flavus* (59.50%), *Alternaria* sp. (58.50%) and *Aspergillus niger* (57.50%). The lowest pre-emergence mortality was observed in the seed inoculated with *Penicillium* sp. (45.75%). Whereas, the lowest pre-emergence mortality (20.50%) was observed in control. .

Table 2: Pathogenicity of isolated fungi on yardlong bean *in vitro*

Treatment no.	Name of fungi	Pre-emergence mortality (%)*	Post-emergence mortality (%)*
1	*Aspergillus niger*	57.50	36.50
2	*Aspergillus flavus*	59.50	40.50
3	*Rhizopus* sp.	68.00	47.75
4	*Penicillium* sp.	45.75	28.50
5	*Alternaria* sp.	58.50	36.50
6	*Sclerotium* sp.	69.00	43.25
7	*Curvularia* sp.	70.00	47.00
8	*Botryodiplodia* sp.	74.50	54.25
9	*Colletotrichum* sp.	74.25	51.75
10	*Fusarium* sp.	65.50	51.50
11	Control	20.50	13.50

*Average of four repetitions

Per cent post-emergence mortality was found higher in the seeds inoculated with *Botryodiplodia* sp. (54.25%) as compared to the rest. Next higher post-emergence mortality was recorded in *Colletotrichum* sp. (51.75%) followed by *Fusarium* sp. (51.50%), *Rhizopus* sp. (47.75%), *Curvularia* sp. (47.00%), *Sclerotium* sp. (43.25%), *Aspergillus flavus* (40.50%), *Aspergillus niger* (36.50%), *Alternaria* sp. (36.50%) and the lowest was observed in *Penicillium* sp. (28.50%). Lowest post-emergence mortality (13.50%) was observed in control. All the isolated pathogens were found to be pathogenic to yardlong bean seeds and reduction in germination of each treatment was caused due to rotting of seeds by inoculated fungi.

3.2.2. Pathogenicity test under in vivo

The surface sterilized apparently healthy seeds of yardlong bean were inoculated with 10 days old culture of each test fungus and 10 seeds for each fungus were sown on previously prepared plastic pots containing sterilized soil. The un-inoculated seed served as control.

The results revealed (Table 3) that inoculation of all mycoflora isolated from seeds of yardlong bean has reduced germination and caused pre and post-emergence mortality as compared to un-inoculated seeds. The highest pre-emergence mortality was recorded in seeds inoculated with *Curvularia* sp. (72.50%) followed by *Colletotrichum* sp. (62.50%) whereas the highest post-emergence mortality was observed in *Fusarium* sp. (47.01%) which was followed by *Sclerotium* sp. (45.03%) and *Botryodiplodia* sp. (44.65%). The lowest pre-emergence mortality (23.75%) and post-emergence mortality (15.46%) was observed in the seeds inoculated with *Aspergillus flavus*. Reduction in germination in each treatment was caused due to rotting of seeds by inoculated fungi.

The result shows similarity with Iyani et al. (2015) who carried out germination test of cowpea seeds inoculated with eight microorganisms in polythene bags along with control and observed that fungi *Fusarium oxysporum* caused the highest reduction in germination (20%) followed by *Aspergillus niger* (24%) compared to control plants which showed 100% germination. However, Chukwu and Enyiukwu (2016) showed the association of *Curvularia lunata*, *Botrydiplodia theobromae*, *Rhizopus stolonifer*, *Aspergillus niger*, *A. flavus* and *Fusarium oxysporum* with the maize seeds and carried out pathogenicity test under *in vivo* condition from which they reported that among all the organisms, *F. oxysporum* showed 90% seedlings affected by the pathogen and 40% died from the infection followed by *B. theobromae* and *C. lunata* which recorded 50% disease incidence and 20% mortality

Table 3: Pathogenicity of isolated fungi on yardlong bean *in vivo*

Treatment no.	Name of fungi	Pre-emergence mortality (%)*	Post-emergence mortality (%)*
1	*Aspergillus niger*	41.25	18.43
2	*Aspergillus flavus*	23.75	15.46
3	*Rhizopus* sp.	44.37	28.17
4	*Penicillium* sp.	32.50	29.41
5	*Alternaria* sp.	39.37	38.18
6	*Sclerotium* sp.	57.50	45.03
7	*Curvularia* sp.	72.50	52.10
8	*Botryodiplodia* sp.	51.87	44.65
9	*Colletotrichum* sp.	62.50	36.92
10	*Fusarium* sp.	45.00	47.01
11	Control	21.87	00.00

*Average of four repetitions

rate, respectively.

3.3. Impact of seed infecting fungi on seed health status with respect to seed germination and seedling vigour

Yardlong bean seeds inoculated with five dominant mycoflora viz., *Fusarium* sp., *Sclerotium* sp., *Botryodiplodia* sp., *Curvularia* sp. and *Colletotrichum* sp. based on their pathogenic nature significantly reduced the seed germination, shoot length, root length and seedling vigour in comparison to control (Table 4).

Seed inoculated with *Fusarium* sp. recorded the lowest seed germination (25.25%) followed by *Sclerotium* sp. (31.75%), *Botryodiplodia* sp. (34.25%), *Colletotrichum* sp. (41.50%) and *Curvularia* sp. (42.50%) over control (81.25%). The results in terms of shoot length, root length with seedling vigour index seed inoculated with *Fusarium* sp. recorded shoot length (6.58 cm), root length (5.11 cm) and seedling vigour index (295.12). Similarly, *Sclerotium* sp. (6.97 cm, 5.85 cm, 407.73), *Botryodiplodia* sp. (7.35 cm, 5.93 cm, 456.64), *Curvularia* sp. (10.04 cm, 7.69 cm, 754.28) and *Colletotrichum* sp. (9.53 cm, 6.16 cm, 651.35) also recorded lower shoot length, root length and seedling vigour index, respectively as compared to control (11.25 cm, 8.09 cm, 1571.57).

The above results are more or less similar with Khodifad and Sharma (2022) who reported that artificial inoculation of soybean seeds with *Fusarium pallidoroseum* caused lower per cent germination (49.00%) over control (95.00%). Rathod et al. (2021) reported that vigour index was 1800 and 2925 in control, while 1000 and 1600 in seeds treated with spore suspension of *A. alternata* and *A. flavus* in blotter paper method and in case of agar plate method seedling vigor index was 2700 and 3420 in control, while 2240 and 2805 in seeds treated with spore suspension of *A. alternata* and *A. flavus.*

Table 4: Impact of seed infecting fungi on seed health status in respect to seed germination and seedling vigour

Seed mycoflora	Seed germination (%)	Seed germination over healthy seed (%)	Shoot length (cm)*	Decrease in shoot length over healthy seed (%)	Root length (cm)	Decrease in root length over healthy seed (%)	Seedling vigour index (SVI)
T_1: *Fusarium* sp.	25.25	68.92	6.58	41.53	5.11	36.92	295.12
T_2: *Sclerotium* sp.	31.75	60.92	6.97	38.00	5.85	27.71	407.73
T_3: *Botryodiplodia* sp.	34.25	57.85	7.35	34.59	5.93	26.72	456.64
T_4: *Curvularia* sp.	42.50	47.69	10.04	10.70	7.69	04.97	754.28
T_5: *Colletotrichum* sp.	41.50	48.92	9.53	15.23	6.16	23.90	651.35
T_6: Control	81.25	---	11.25	---	8.09	---	1571.57
SEm±	0.74		0.16		0.16		16.53
CD (*p*=0.05)	2.19		0.47		0.48		49.11
CV%	3.44		3.66		4.94		4.79

*Average of four repetitions

4. Conclusion

This study identified ten yardlong bean seed-associated fungi, including *Aspergillus niger*, *A. flavus*, *Penicillium* sp., *Rhizopus* sp., *Fusarium* sp., *Colletotrichum* sp., *Curvularia* sp., *Alternaria* sp., *Botryodiplodia* sp., and *Sclerotium* sp. Five dominant pathogens were tested for their effects on seed germination, shoot length, root length, and SVI. *Fusarium sp.* caused the highest reduction with germination 25.25 per cent, shoot length 6.58 cm, root length 5.11 cm and SVI 295.12, compared to control (81.25%, 11.25 cm, 8.09 cm, 1571.57).

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