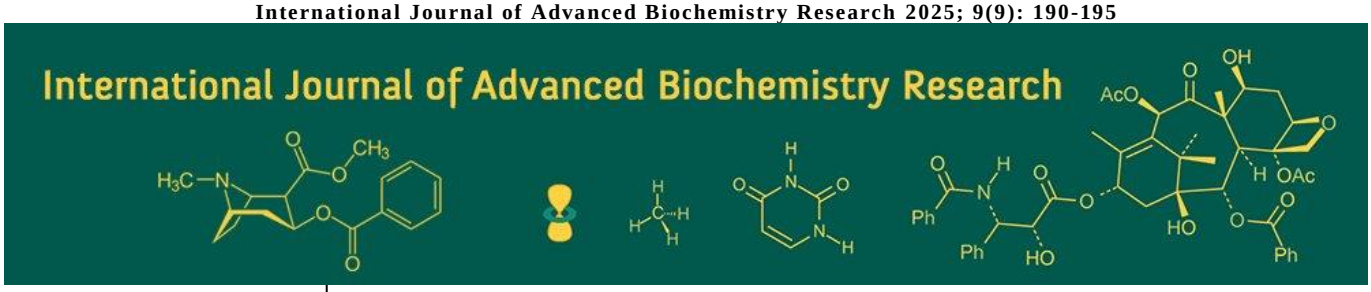

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(9): 190-195
www.biochemjournal.com
Received: 09-07-2025
Accepted: 11-08-2025

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Impact of seed borne mycoflora on germination and seedling vigour of black gram [*Vigna mungo* (L.) Hepper]

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i9c.5560

Abstract
The present study aimed to investigate the impact of seed borne mycoflora on black gram [*Vigna mungo* (L.) Hepper]. Seed germination and subsequent seedling vigour are important factor for the influencing crop establishment and yield potential. Six varieties of black gram seed sample *viz*-Indira urd-1, Pratap, KU-96, TPU-4, TIU-22, Local varieties and seven seed borne mycoflora *viz-Fusarium* spp., *Alternaria* spp., *Trichoderma* spp., *Chaetomimum* spp., *Curvularia* spp., *Penicillium* spp. and *Cladosporium* spp. were selected for the experiment. A pot experiment was conducted to study the effect of seed borne mycoflora on seed germination and seedling vigour of black gram. The results revealed that, among seed inoculation techniques maximum reduction in seedling index over control was observed in *Chaetomium* spp. (58.93%) Overall, increased seedling vigour of black gram varieties by *Trichoderma* spp. inoculated seed lots was recorded (16.91%). Among soil inoculation techniques maximum reduction in seedling index overcontrol was observed in *Curvularia* spp. (75.23%) inoculated seedlings and over all increased seedling vigour of black gram varieties was observed in *Trichoderma* spp. (21.14%) inoculated seedlings.

Keywords: Black gram, seedling vigour index, seed borne mycoflora, germination

1. Introduction
Black gram is one of the most important pulse crops grown during both the *Kharif* and *Rabi* seasons in India. It is a staple legume widely consumed across the country and plays a vital role in sustainable agriculture by enhancing soil fertility through biological nitrogen fixation. As a short-duration crop, black gram is grown throughout India, often cultivated as a mixed crop, cash crop, or in sequential cropping systems. It is commonly grown as a single crop after the rice harvest and before or after the harvest of other summer crops in semi-irrigated and dryland conditions.

Black gram is highly nutritious, containing 25 grams of protein and 58.99 grams of carbohydrates per 100 grams of seeds. It is also rich in phosphoric acid, phosphorus (3.85 mg/100g), iron (10.2 mg/100g), thiamin (0.42%/100g), riboflavin (0.20 mg/100g), niacin (2 mg/100g), and vitamin C (3 mg/100g), (Shakuntala Manay and M Shadaksharaswamy, 1987) [12].

The productivity of black gram is less as compare to national productivity region because the crop is exposed to several biotic factors i.e. insect disease and tools etc. Among the biotic factors, crop is affected by many diseases i.e. Cercospora leaf spot caused by *Cercospora canescens*, Anthracnose caused by *Colletotrichum lindemuthianum*, powdery mildew caused by *Erysiphe polygoni*, leaf crinkle disease caused by Leaf Crinkle Virus, and a new disease, *Rhizoctonia bataticola* causes aerial blight and dry root rot of black gram, which reduces the yield up to 60% (Patil *et al*. 2012) [7]. The pathogen is transmitted via plants, and seed-to-seedling transmission has been reported where infected seeds are used and there pathogens affects the root, stem, buds, petiols, leaves, pods, and seeds (Khanzada *et al*. 2002) [6].

2. Material and Methods
The current study was conducted in the Department of Plant Pathology, Collage of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, between, 2020-2021.

Six varieties of black gram seed sample *viz*-Indira urd-1, Pratap, KU-96, TPU-4, TIU-22, Local varieties and seven seed borne mycoflora *viz-Fusarium* spp., *Alternaria* spp., *Trichoderma* spp., *Chaetomimum* spp., *Curvularia* spp., *Penicillium* spp. and *Cladosporium* spp. were selected for the experiment was conducted to study the effect of seed-borne mycoflora on seed germination and seedling vigour of black gram.

2.1 Effect of seed borne mycoflora seedling vigour of black gram seeds (Seed inoculation technique)

In this investigation, firstly 50 seeds of each variety selected randomly and surface sterilized with 1.0% NaOCl solution for 30 seconds and immediately seeds was washed with sterile distilled water. Thereafter seeds were rolled separately in sporulating cultures of detected mycoflora A. *niger, A. flavus, Fusarium sp., Alternaria alternata, Rhizopus sp*. etc. surviving on PDA in petri plates. The mycoflora rolled seeds were sown in sterilized soil in pots (12x12). The seed without rolling in any mycoflora were sown and treated as control. Ten seeds were seeded in each pot and replicated 5 times for each variety. Pots were watered on a regular intervals up 21 days after seeding. The observation were noted for germination percentage, root length, and shoot length of each inoculated and control pots to determine the seedling vigour index. The shoot length was measured from the base of shoot to upper most tip of leaf. For measuring the root length, plant was carefully uprooted first, gently washed and carefully placed on clean transparent glass piece. The length of root system was measured from collar region to longest tip of root. The seedling vigour index was calculated by using the following formula given by Abdul-Baki and Anderson (1973) [1].

Seedling vigour index = (Mean shoot length + Mean root length) X Germination percent

2.2 Effect of seed borne mycoflora on for Gemination and seedling vigour of black gram seeds (Soil inoculation technique)

The seed-borne fungi detected on different varieties of black gram were grown separately on PDA and multiplied in mass on wheat grains. Wheat grains were soaked in plain water for 6 hrs then they were boiled till they become soft but not rupture. Thereafter water was cleaned from grain and spreader on muslin cloth to remove the excess water. After removing of excess water, 1% gypsum and 1% CaCO3 was added and filled in conical flask (500ml) and plugged with non-absorbent cotton. These flask were sterilized at 20 lbs/sq inch for 20 min. Thereafter flask were cooled down at room temperature. These sterilized grains were aseptically inoculated at individually with detected seed borne mycoflora. The inoculated flask were incubated at 25±1 °C and observed regularly for any kind of contamination and those flask were showed any type of contamination were substrate by individual fungi was used for inoculation in soil. The mass multiplied culture of each fungi was thoroughly mixed in pre sterilized soil (10g/pot) and filling in pots (12x12). Thereafter watery was done to just wet the soil. Then pots were placed in open for 72 hours. After 3 days establishment of mycoflora before sowing of seeds. Seeds of different varieties of black gram were surface sterilized (1.0 percent NaOCl) before sowing followed by washed 3 times with sterile distilled water and sown in inoculated pot. Surface sterilized seeds were also sown in sterilized un-inoculated soil which were served as control. Pots were watered on a regular basis. The observation were taken for seedling growth in term of seedling vigour index after 21 days of sowing as described earlier.

3. Results and Discussions

3.1 Effect of seed borne mycoflora on seedling vigour of black gram seeds (Seed inoculation technique)

It is depicted from data presented in table that seedling vigour was markedly reduced by some of the seed borne mycoflora when evaluated by seed inoculation technique. Overall impact in reducing seedling vigour index was shown by *Aspergillus flavus* across all 6 varieties as compared to that of control. Maximum reduction in seedling vigour index of TIU-22 variety was caused by *Chaetomium* spp. (58.93%) followed by *Cladosporium* spp. (19.69%), *Curvularia* spp. (12.95%), *Fusarium* sp. (11.92%), *Alternaria* spp. (7.12%). In the seed sample of local variety, reduction was maximum by *Curvularia lunata* (68.36%) followed by and *Fusarium* spp. (56.70%), *Cladosporium* spp. (36.74%), *Chaetomium* spp. (33.16%), *Alternaria* spp. (22.16%). Reduction in the seedling vigour index of Indira urd-1 variety reduction was maximum by *Fusarium* spp. (42.31%) followed by *Chaetomium* spp. (27.60%), *Cladosporium* spp. (23.49%) and *Alternaria* spp. (7.58%). In the seed lot of Pratap-1 variety, reduction was maximum by Fusarium sp. (48.52%) followed by *Chaetomium* spp. (41.52%), *Curvularia s*pp. (23.10%), *Alternaria* spp. (7.13%) and *Cladosporium* spp. (1.04%). KU-96 variety reduction was maximum by *Chaetomium* spp. (47.76%) followed by *Alternaria* spp. (13.92%). *Fusarium* sp. (6.12%) and *Cladosporium* spp. (4.57%). In the seed sample of TPU-4 variety, reduction was maximum by *Curvularia* spp. (64.57%) followed by *Fusarium* spp. (23.23%), *Alternaria* spp. (7.79%) *Cladosporium* spp. (6.16%) and *Chaetomium* spp. (2.23%). It was observed that *Chaetomium globosum* (35.2%), recorded mean maximum reduction followed by *Fusarium* sp. (31.46%), *Curvularia lunata* (28.14%), *Cladosporium* spp. (15.28%) and *A. alternata* (12.13%). 78 Overall, increased seedling vigour of black gram varieties by *Trichoderma* spp. inoculated seed lots was recorded (16.91%) followed by *Penicillium* spp. (7.59%). Seeds of black gram varieties inoculated with *Trichoderma* spp. and *Penicillium* spp. showed reverse trends among all the mycoflora. *T. viride* and *Penicillium* sp. may exhibits plant growth promoting activities, hence it increased seedling vigour of black gram varieties as compared to the control and other seed associated different mycoflora.

Hence, it was proven that the isolated seed associated mycoflora were pathogenic to the black gram seeds and detected seed transmissible in present study. Chaudhary et al. (2017) recorded the reduction in seed germination and plant vigour index due to the various fungal flora in pigeon pea. Pradhan *et al*. (2017) [9] evaluated that the overall impact of *Rhizopus* sp. and *Fusarium* sp. in the seedling vigour of mungbean varieties. Seed associated mycoflora were recorded to reduce the germination, root and shoot length significantly in different legumes also. Saurabh and Singh (2020) [11] conducted a pot experiment *in vitro* to find out the effect of *Aspergillus flavus* in the germination of black gram seed and recorded 4.35% germination in inoculated seeds as compare to control. Sahu (2020) [10]

reported that germination, seedling length and seedling vigour in lentil seeds was drastically decreased by seed associated mycoflora when evaluated by seed inoculation method. Findings of all above mentioned workers supports the findings of present study.

(i) Mycoflora (seed inoculation) (ii) Mycoflora (soil inoculation)

(iii) Bio-control (iv) *Trichoderma viride*

(v) Fungicide (vi) Control

Fig 1: Effect of seed borne mycoflora on seedling vigour of black gram varieties

Table 1: Effect of seed borne mycoflora on seedling vigour of black gram varieties (seed inoculation technique) and% Increase or decrease over control

S. No.	Mycoflora	Seedling vigour index and% Increase or Decrease over control												Mean seedling vigour index	Mean increase / decrease over control
		Indira urd-1		Pratap-1		KU-96		TPU-4		TIU-22		Local variety			
		SVI	%Inc, Dec.	SVI	%Inc, Dec.	SVI	%Inc, Dec.	SVI	%Inc., Dec.	SVI	%Inc., Dec.	SVI	%Inc., Dec.		
1	*Fusarium* spp.	648.30	-42.31	547.17	-48.52	956.80	-6.12	935.23	-23.23	875.68	-11.92	469.78	-56.70	738.82	-31.46
2	*Alternaria* spp.	1038.23	-7.58	987.18	-7.13	877.35	-13.92	1123.34	-7.79	923.38	-7.12	844.65	-22.16	965.68	-12.13
3	*Trichoderma* spp.	1354.23	+20.53	1328.55	+24.98	1425.84	+39.88	1294.70	+6.27	1046.32	+5.23	1135.40	+4.62	1264.17	+16.91
4	*Chaetomimum* spp.	813.38	-27.60	621.56	-41.52	532.43	-47.76	1191.10	-2.23	408.25	-58.93	725.32	-33.16	715.34	-35.2
5	*Curvularia* spp.	*	*	817.41	-23.10	1020.36	0.10	431.53	-64.57	865.43	-12.95	343.25	-68.36	579.66	-28.14
6	*Penicilliumspp.*	1234.22	+9.85	1085.43	+2.11	1193.54	+17.09	1255.74	+3.07	1036.53	+4.25	1185.12	+9.21	1165.07	+7.59
7	*Cladosporium* spp.	859.57	-23.49	1051.84	-1.04	972.58	-4.57	1143.18	-6.16	798.46	-19.69	686.42	-36.74	918.67	-15.28
8	Control	1123.48		1063.00		1019.26		1218.30		994.25		1085.17		1083.91	

* Not germinated seed

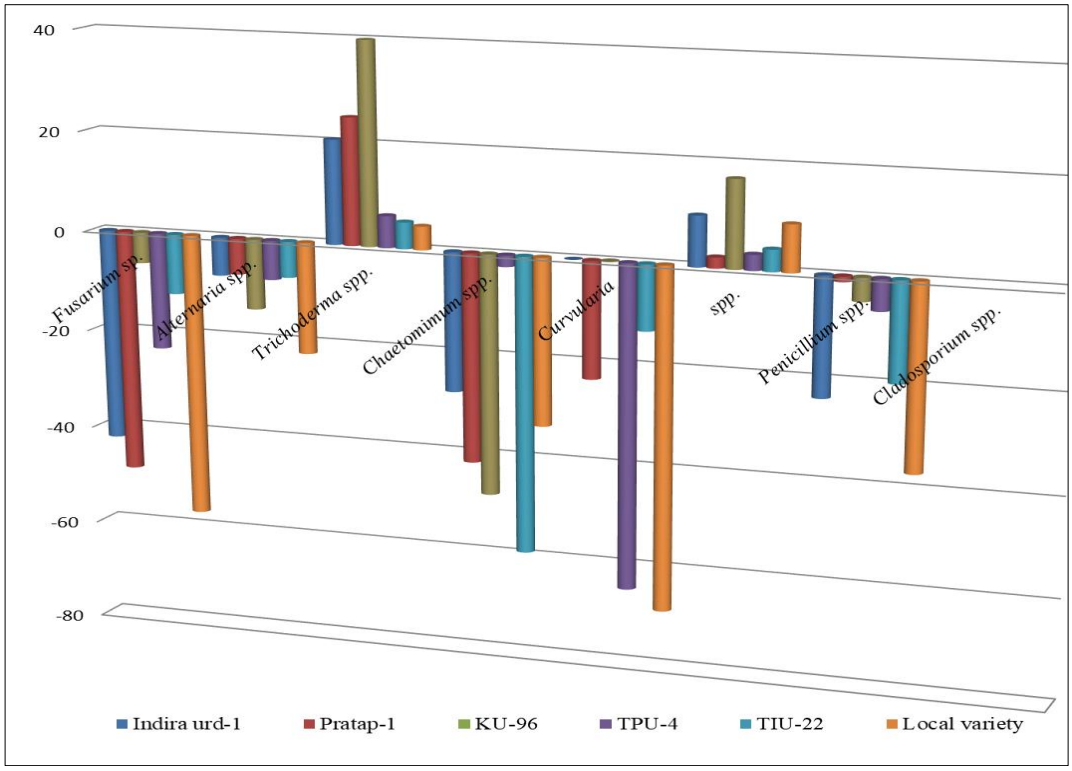

Fig 2: Effect of seed borne mycoflora on seedling vigour of black gram varieties (seed inoculation techniques (% Increase or Decrease over control)

3.2 Effect of seed borne mycoflora on seedling vigour of black gram seeds (Soil inoculation technique)

Soil inoculation technique was used to know the effect of seed borne mycoflora on seedling vigour index and data presented in table 4.3.2. It was clear from the table that *Curvularia* spp. reduced the seedling vigour index maximum i.e. 49.67% irrespective of seed lots followed by *Fusarium* spp. (46.75%) and *Chaetomimum* spp. (41.22%) in comparison to control. Minimum reduction in seedling vigour index of all black gram varieties were recorded in soil inoculation with *Alternaria* spp. (37.89%) followed by *Cladosporium* sp. (31.09%). In Indira urd-1 variety, maximum reduction in seed lot was recorded by *Curvularia* spp. (75.23%) which was closely followed by *Fusarium* spp. (61.85%). *Chaetomimum* spp. (36.51%), *Cladosporium* spp. (32.40%), *Alternaria* spp. (34.29%). In Pratap-1 variety, maximum reduction in seedling vigour index was recorded in *Fusarium* spp. (67.34%) followed by *Alternaria* spp. (66.06%), *Curvularia* spp. (41.52%), *Chaetomimum* spp. (41.15%), *Cladosporium* spp. (4.73%). *Fusarium* sp. reduces maximum vigour index (65.37%) followed by *Chaetomimum* spp. (61.16%), Curvularia spp. (59.04%), *Alternaria* spp. (33.49%), *Cladosporium* spp. (28.57%) in KU-96 variety. In TPU-4 variety reduction in seedling vigour index was maximum by *Curvularia* spp. (59.65%) followed by *Cladosporium* spp. (51.39%), *Alternaria* spp. (44.26%), *Chaetomimum* spp. (18.25%), *Fusarium* spp. (16.37%). In TIU-22 variety reduction in seedling vigour index was maximum by *Chaetomimum* spp. (61.65%) followed by *Cladosporium* spp. (29.62%) and *Curvularia* spp. (23.31%) *Fusarium* spp. (22.13%), *Alternaria* spp. (17.88%). *Fusarium* spp. reduces maximum seedling vigour index (47.49%) followed by *Cladosporium* spp. (39.88%) *Curvularia* spp. (38.88%) *Chaetomimum* spp. (32.87%), *Alternaria* spp. (31.36%) in local variety. In case of *Trichoderma* and *Penicillium* spp. increased seedling vigour (21.14%), (19.11%) of black gram varieties as compared to control was observed, while decreased seedling vigour as compared to control was observed in soil inoculated with other mycoflora.

Table 2: Effect of seed borne mycoflora on seedling vigour of black gram varieties (soil inoculation technique)

S. No	Mycoflora	Seedling vigour index% Increase or Decrease over control												Mean seedling vigour index	Mean increase/ decrease over control
		Indira urd-1		Pratap-1		KU-96		TPU-4		TIU-22		Local variety			
		SVI	%Inc., Dec.	SVI	%Inc., Dec.	SVI	%Inc., Dec.	SVI	%Inc., Dec.	SVI	%Inc., Dec.	SVI	%Inc., Dec.		
1	*Fusarium* spp.	428.50	-61.85	347.18	-67.34	456.80	-65.37	935.23	-16.37	875.68	-22.13	569.78	-47.49	602.19	-46.75
2	*Alternaria* spp.	738.23	-34.29	387.18	-66.06	877.35	-33.49	623.34	-44.26	923.38	-17.88	744.85	-31.36	715.72	-37.89
3	*Trichoderma* sp.	1334.53	18.78	1328.25	24.92	1525.14	15.60	1284.90	14.89	1416.32	25.94	1375.40	26.74	1377.42	+21.14
4	*Chaetomimum* spp.	713.28	-36.51	625.61	-41.15	512.33	-61.16	941.20	-18.25	431.21	-61.65	728.39	-32.87	654.17	-41.22
5	*Curvularia* spp.	278.20	-75.23	617.41	-41.92	540.26	-59.04	451.23	-59.65	862.33	-23.31	663.15	-38.88	568.76	-49.67
6	*Penicillium* spp.	1544.57	37.48	1295.53	21.85	1493.64	13.21	1265.84	13.19	1346.73	19.75	1185.12	9.21	1355.23	+19.11
7	*Cladosporium* spp.	759.37	-32.40	1021.84	-4.73	942.28	-28.57	543.58	-51.39	791.36	-29.62	652.32	-39.88	785.12	-31.09
8	Control	1123.48		1063.20		1319.26		1118.30		1124.55		1085.17		1138.99	

* Not germinated seed

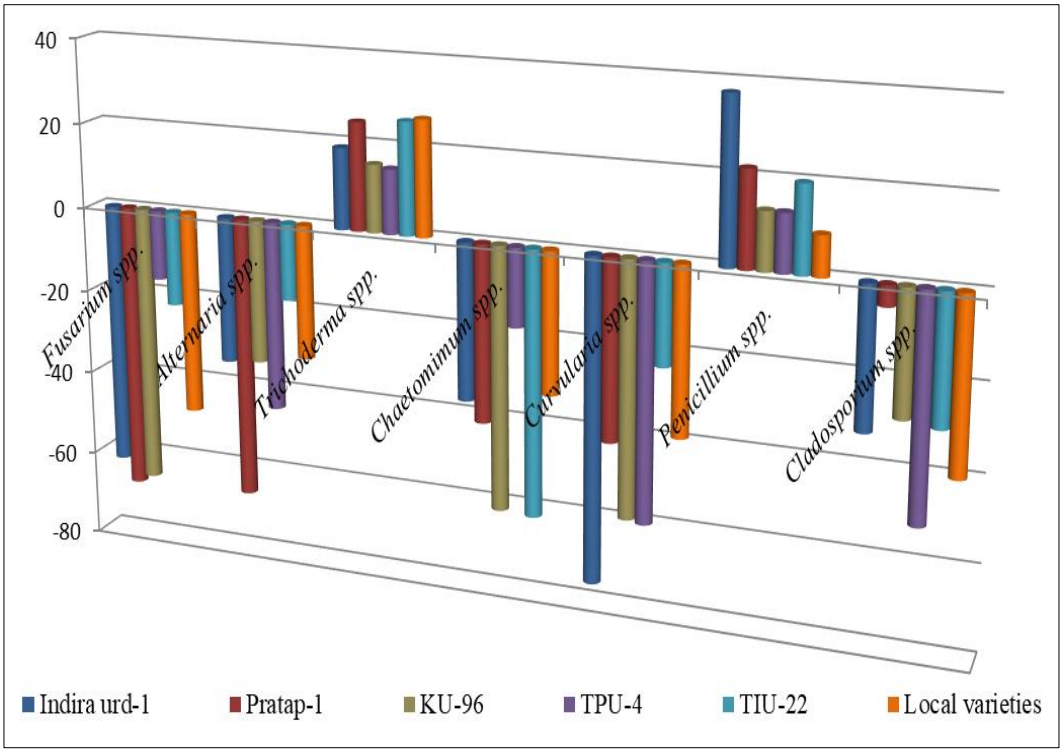

Fig 3: Effect of mycoflora on seedling vigour of black gram varieties (soil inoculation technique), (% Increase or Decrease over control

Similar result was also observed by Kandhare (2014) [5] examined the effect of seed borne mycoflora on seed health by germination 82 and seedling emergence technique. The mycoflora *A. Niger* and *Drechslera tetramera* affected adversely to seedling emergence. Chaudhary *et al*. (2016) Seed associated fungi and their culture filtrate caused reduction in germination percentage and growth of seedlings as compared to the untreated check. Minimum seed germination were observed in A. Niger treatment in both culture filtrate (43.00%) and seed inoculation (56.00%). Pradhan (2017) [9] used for inoculation in mungbean were *A. flavus, A. fumigatus, A. Niger, Alternaria sp.* overall impact in decreasing seedling vigour index. Sahu (2020) [10] recorded that the seedling vigour of lentil was distinctly decreased by some of the seed borne mycoflora when evaluated by soil inoculation techniques.

4. Summary

The study highlights the impact of seed-borne mycoflora on seedling vigor in black gram using seed inoculation and soil inoculation techniques. *Chaetomium spp.* and *Curvularia spp.* caused initial rotting symptoms and were confirmed to be pathogenic and seed-transmissible in black gram. *Trichoderma viride* was associated with increased seedling vigor in seed inoculation trials, even in the presence of other mycoflora.

This suggests a differential impact of mycoflora on seedling vigor and highlights the potential of *Trichoderma viride* as a beneficial agent for enhancing vigor.

5. Conclusion

Seedling vigour was markedly reduced by some of the associated seed borne mycoflora when analysed by seed inoculation and soil inoculation technique. In seed inoculation technique, *Chaetomium* spp. shows overall impact irrespective of seed lots followed by *Fusarium* spp. whereas in soil inoculation technique, *Curvularia* spp. reduce the vigour index maximum irrespective of seed lots followed by *Fusarium* spp. and *Chaetomimum* spp. in comparison to that of control. Seedling showed initial rotting type symptoms produced by *Chaetomium* spp. and *Curvularia* spp. were found to be pathogenic to black gram and seed transmissible in nature. In case of *Trichoderma viride*, increased seedling vigour as compared to the control was observed in seed inoculation with other mycoflora

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