Effect of Seed-Borne Mycoflora on Seed Germination and Seedling Vigour of Soybean (*Glycine max* L.)

ABSTRACT

The present study aimed to investigate the impact of seed-borne mycoflora on soybean (*Glycine max* L.). Seed germination and subsequent seedling vigour are important factors for influencing crop establishment and yield potential. Six varieties of soybean seed samples *viz*. JS 95-60, JS 97-52, RSC 10-46, RSC 10-71, CG Soya-1 and seven seed-borne mycoflora *viz*. *Fusarium* spp., *Alternaria spp*., *Colletotrichum spp., Cladosporium spp., Macrophomina spp., Penicillium spp*. and *Trichoderma 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 soybean. The results revealed that, among seed inoculation techniques maximum reduction in seedling vigour index over control was observed in *Colletotrichum* spp. (27.24%) inoculated seedlings and overall increased seedling vigour of soybean varieties was observed in *Trichoderma spp.* (26.52%) inoculated seedlings. Among soil inoculation techniques maximum reduction in seedling vigour index over control was observed in *Macrophomina spp.* (38.41%) inoculated seedlings and overall increased seedling vigour of soybean varieties was observed in *Trichoderma spp.* (24.40%) inoculated seedlings.

Keywords: Soybean; seedling vigour; seed-borne mycoflora; seed germination.

1. INTRODUCTION

Soybean (*Glycine max* L.) holds a vital position in global agriculture as a significant source of protein, oil, and other essential nutrients for both humans and animals. Soybean is popularly known as 'Golden bean' or 'Miracle bean' and 'Wonder bean' of the 20th century because of its characters and usage. It is the most common oilseed crop grown mostly in the rainy season. Soybean seeds have a greater nutritional value it is a major source of protein and vegetable oil. It contains 40-42% proteins, 20-22% oil, 21% starch, vitamins- A, B, C, D & K beside essential amino acids like lysine (5%) and a small amount of calcium, phosphorous, magnesium and iron [1]. Soybean being a nutritious crop plays a significant role in overcoming problems of food and nutritional insecurity especially in developing countries [2]. It is the most important and least expensive protein source produced worldwide [3]. It has been reported that the Indian continent is the secondary Centre for domestication of the crop after China [4]. The crop enhances soil fertility and economizes crop production by minimizing the regular rate of nitrogen fertilizer [5]. Disease pressure appears to be increasing as yield losses are seen to increase over time [6]. The successful establishment of soybean crops heavily relies on the successful germination of seeds and subsequent vigorous seedling growth. Seed germination and seedling vigour play a pivotal role in determining the crop's stand establishment, early growth rate, and ultimate yield potential. However, these processes are influenced by a multitude of factors, including the presence of various microorganisms associated with seeds, collectively referred to as seed-borne mycoflora.

Seed-borne mycoflora consist of a diverse array of fungi that can be present externally on the

seed coat or internally within the seed tissues. These fungi have the potential to either facilitate or impede the germination and early growth stages of plants. The interactions between seed-borne mycoflora and plants are intricate and can vary depending on the species of fungi, the specific host plant, and the prevailing environmental conditions. Understanding the dynamics of these interactions is of paramount importance for optimizing seed health management and subsequently enhancing crop productivity.

2. MATERIALS AND METHODS

The present investigation was conducted in the Department of Plant Pathology, College of Agriculture, I.G.K.V., Raipur (C.G) during the year 2020-21. Six varieties of soybean seed samples *viz*. JS 95-60, JS 97-52, RSC 10-46, RSC 10-71, CG Soya-1 and seven seed-borne mycoflora *viz*. *Fusarium* spp., *Alternaria spp*., *Colletotrichum spp., Cladosporium spp., Macrophomina spp., Penicillium spp*. and *Trichoderma 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 soybean.

2.1 Seed Inoculation Technique for Germination and Seedling Vigour of soybean seeds

For this investigation, healthy soybean seeds were surface sterilized by 1.0 per cent NaOCl for 5 minutes and then rinsed in tap water 3 times. The seeds were rolled on 7 to 10 days old sporulating culture of individually detected mycoflora thriving on PDA (potato dextrose agar) in petri plates. The rolled seeds were grown in pots (35.6x30x28.6cm LxWxH) containing sterilized soil. A set of control was also kept with surface-sterilized seeds without rolling on a culture of mycoflora, sown in sterilized soil pots. The pots were watered regularly. The seedling vigour index was used to examine seedling growth 21 days after seeding. The germination percentage, shoot length and root length were recorded to calculate the seedling vigor index of each inoculated and control seed sample. The length of the shoot was measured from the base of the shoot to the uppermost tip of the leaf. To measure the root length, the plant was uprooted first carefully, gently washed and placed carefully on a clean transparent glass piece. The root length was measured from the collar region to the end of the longest tip of the root. Abdul-Baki and Anderson [7], provided the following formula for calculating the seedling vigour index.

> Seedling vigour index = (Mean shoot length + Mean root length) * Germination percentage

2.2 Soil Inoculation Technique for Germination and Seedling Vigour of Soybean Seeds

All of the seed-associated fungi detected by using different seed testing methods were cultivated and propagated individually in sterilized wheat grain substrate. Individually, each substrate was injected with a 7-day-old fungus culture. The inoculated substrate was incubated for 10 days at 25± 1˚C. To avoid clumping substrate was shaken every day. The (35.6x30x28.6cm LxWxH) size pots were filled with sterilized soil and inoculated by fungal inoculum. For soil inoculation, the upper 4 cm layer of soil was thoroughly mixed with culture grown in wheat medium @ 10g/pots and watered to just wet the inoculated. For proper soil infection and establishment of mycoflora before sowing of soybean seeds, pots were kept in a glass house for 72 hours. Before sowing, seeds were surface-sterilized 1.0% NaOCl followed by 3 washing with sterilized “distilled water and were sown in inoculated soil @ 10 seeds per pot. A set of control was also kept with surface-sterilized seeds sown in sterilized” uninoculated soil. At regular intervals, pots were watered. The seedling growth was recorded in terms of seedling vigour index 21 days after sowing as described earlier.

3. RESULTS AND DISCUSSION

3.1 Seed Inoculation Technique for Germination and Seedling Vigour of Soybean Seeds

It was depicted from the data presented in the table that seedling vigour was noticeably reduced by some of the seed-borne mycoflora when examined by seed inoculation technique. The overall impact of reducing seedling vigour index was shown by *Colletotrichum spp*. across all six varieties evaluated as compared to that of control.

Maximum reduction in seedling vigour index over control was observed in local variety which was caused by *Colletotrichum* spp. (27.24%) followed by *Macrophomina* spp.

(21.87%) and *Fusarium* spp. (17.95%). In the seed lot of local variety, seedling vigour reduction was maximum by *Colletotrichum spp.* (49.07%) followed by *Fusarium* spp*. (*37.48%). Whereas, *Colletotrichum* spp. caused maximum (27.24%) reduction in seedling vigour index of different varieties of soybean seed lots i.e., local variety (49.07%), RSC 10-46 (29.48%), CG Soya-1 (25.06%), RSC 10-71 (24.15%), JS 95-60 (19.86%) and JS 97-52 (15.83%).

Overall, increased seedling vigour of soybean varieties by *Trichoderma* spp. inoculated seed lots were recorded (26.52%) followed by *Penicillium* spp. (15.11%). Seeds of soybean varieties inoculated with *Trichoderma* spp. and *Penicillium* spp., showed reverse trends among all the mycoflora. *Trichoderma* spp*.* and *Penicillium* spp*.* may exhibit plant growth-promoting activities, hence it increased seedling vigour of soybean varieties as compared to the control and other seed-associated different mycoflora.

Hence, it was proven that the isolated seed-borne mycoflora were pathogenic to the soybean seeds and detected seed transmissible in the present study. Pradhan et al. [8] detected that the overall impact of *Rhizopus* spp. and *Fusarium* spp. in the seedling vigour of mung bean varieties. Seed-borne mycoflora were recorded to reduce the germination, root and shoot length significantly in different legume crops. Kesharwani et al. [9] also detected the seedling vigour of different varieties of pea was distinctly decreased by some of the seed-associated mycoflora when examined by the seed inoculation techniques. Pradhan [10] in Indian bean varieties reported that germination, seedling length and seedling vigour were drastically decreased by seed-associated mycoflora when evaluated by the seed inoculation method and Sahu [11] detected some seed-associated fungal flora when exanimated by seed inoculation technique. The overall effect in reducing seedling vigour index was shown by *A. flavus* among all 6 varieties evaluated as compared to that of control workers supporting the findings of the present study.

3.2 Soil Inoculation Technique for Germination and Seedling Vigour of Soybean Seeds

Soil inoculation technique was used to know the effect of seed-associated mycoflora on seedling vigour index and data presented in table. It was clear from the table that *Macrophomina* spp. reduced the seedling vigour index maximum i.e., (38.41%) irrespective of seed lots followed by *Colletotrichum* spp. (29.60%) and *Fusarium* spp. (28.95%) in comparison to control. A minimum reduction in the seedling vigour index of all soybean varieties was recorded in soil inoculation with *Cladosporium* spp. (25.87%) followed by *Alternaria* spp. (26.06%). In JS 97-52 variety, the maximum reduction in seed lot was recorded by *Macrophomina* spp. (56.53%), followed by JS 95-60 (46.28%), RSC 10-46 (37.08%), CG Soya-1 (34.29), Local Variety (28.82%) and RSC 10-71 (28.01%).

Overall, increased seedling vigour of soybean varieties by *Trichoderma* spp. inoculated seed lots were recorded seedling vigour (24.40%) followed by *Penicillium* spp. (10.16%). Seeds of soybean varieties inoculated with *Trichoderma* spp. and *Penicillium* spp. showed reverse trends among all mycoflora. *Trichoderma* spp. and *Penicillium* spp. may exhibit plant growth-promoting activities, hence it increased seedling vigour of soybean varieties as compared to the control and other seed-associated different mycoflora. Kesharwani et al. [9] reported that the seedling vigour of pea varieties was distinctly decreased by some seed-borne fungal pathogens when recorded by soil and soil inoculation techniques. A pot experiment was conducted to evaluate the effect of seed-borne fungal pathogens on seedling vigour by seed inoculation method. Vasava et al. [12] recorded the seed germination, seedling length and seedling vigour index of different varieties of cowpea were drastically reduced by all seed-borne mycoflora except *M. phaseolina* and *A. alternata*. Pradhan [10] found that the seedling vigour reduction by seed-borne fungal flora when evaluated by soil inoculation technique. The overall highest reduction in seedling vigour index was observed in *Aspergillus niger* across all 6 Indian bean varieties evaluated as compared to control.

These methods being quick and simple and can be used in routine pathogenicity tests of fungal pathogens, both methods were of equal importance. It was recorded that these fungi reduced the per cent seed germination and seedling vigour as reported by different workers supports the findings of the present study.

Phytotoxic metabolites are produced by a variety of plant pathogenic fungi. When the pathogen is seed-borne, the importance of producing toxic metabolites is magnified because they either

inhibit seed germination or harm seedling development. The development of aflatoxin in good grains interferes with protein synthesis by inhibiting the absorption of amino acids into protein, resulting in the non-germination of embryos. Aflatoxin causes seed germination, hypocotyl or root elongation, and root elongation in growing seeds to be inhibited confers the findings of the present study.

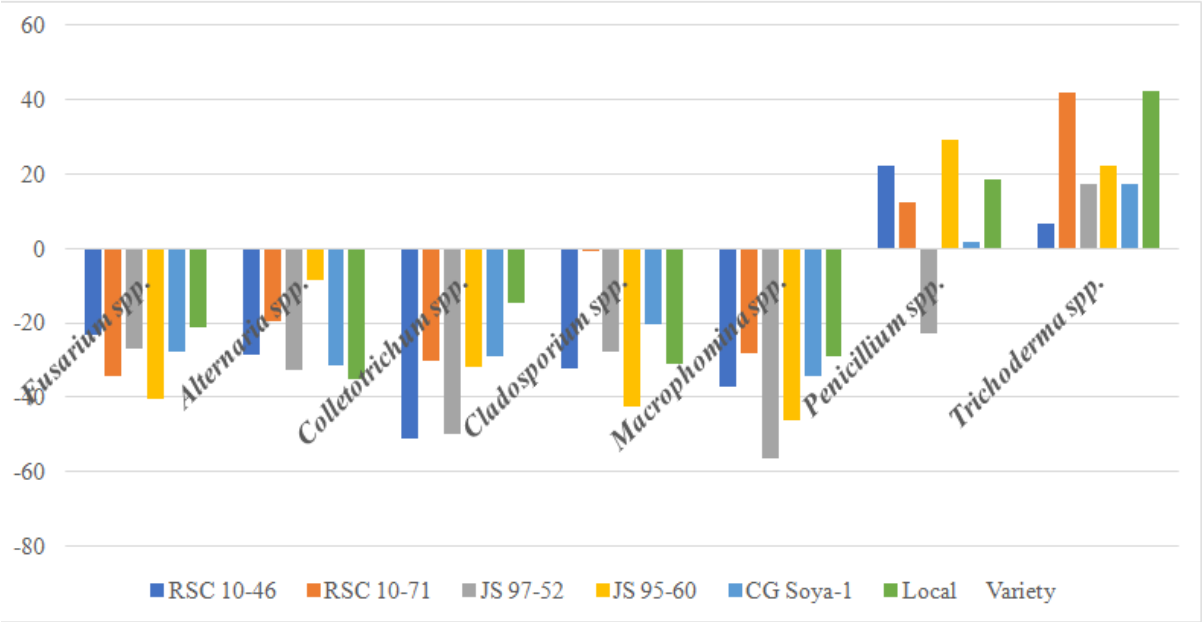

Fig. 1. Effect of seed-borne mycoflora on seedling vigour of soybean seeds (seed inoculation technique)

Table 1. Effect of seed-borne mycoflora on seedling vigour of soybean seeds (seed inoculation technique)

Mycoflora	Seedling vigour index (% Increase (+) or decrease (-))												Mean Seedling vigour index	Mean increase/ decrease over control
	RSC 10-46		RSC 10-71		JS 97-52		JS 95-60		CG Soya-1		Local Variety			
	SVI	% I or % D	SVI	% I or % D	SVI	% I or % D	SVI	% I or % D	SVI	% I or % D	SVI	% I or % D		
Fusarium spp.	917.23	-22.69	973.91	-14.94	915.70	-17.77	1022.48	-4.89	1320.51	-11.52	571.40	-35.94	998.41	-17.95
Alternaria spp.	876.97	-26.08	1036.10	-9.51	826.80	-25.75	910.49	-15.31	1249.30	-16.29	872.10	-2.23	1042.24	-15.86
Colletotrichum spp.	836.62	-29.48	868.50	-24.15	937.26	-15.83	861.51	-19.86	1118.50	-25.06	454.25	-49.07	882.21	-27.24
Cladosporium spp.	1099.86	-7.30	890.00	-22.27	1060.42	-4.77	975.88	-15.31	1196.52	-19.82	810.04	-9.19	1055.45	-13.11
Macrophomina spp.	736.00	-37.96	993.24	-13.25	785.87	-29.43	933.91	-13.13	1394.52	-6.56	616.32	-30.90	976.02	-21.87
Penicillium spp.	1194.50	+0.64	1211.20	+6.65	1490.00	+33.79	1314.92	+22.30	1602.90	+7.40	1069.80	+19.92	1312.55	+15.11
Trichoderma spp.	1642.19	+38.40	1437.39	+25.53	1586.74	+42.48	1232.59	+14.64	1846.99	+23.75	1003.78	+12.52	1458.28	+26.22
Control	1186.48		1145.03		1113.65		1075.12		1492.45		892.05		1105.92	

**SVI- Seedling vigour index*

**% I or % D - (% Increase (+) or decrease (-)*

Table 2. Effect of seed borne mycoflora on seedling vigour of soybean seeds (soil inoculation technique)

Mycoflora	Seedling vigour index (% Increase (+) or decrease (-))												Mean Seedling vigour index	Mean increase/ decrease over control
	RSC 10-46		RSC 10-71		JS 97-52		JS 95-60		CG Soya-1		Local Variety			
	SVI	% I or % D	SVI	% I or % D	SVI	% I or % D	SVI	% I or % D	SVI	% I or % D	SVI	% I or % D		
Fusarium spp.	919.32	-23.21	837.53	-34.42	1067.91	-26.92	759.26	-40.30	1215.20	-27.76	683.27	-21.09	913.74	-28.95
Alternaria spp.	854.27	-28.64	1027.05	-19.58	981.21	-32.85	1163.21	-8.55	1152.55	-31.48	560.12	-35.31	956.40	-26.06
Colletotrichum spp.	585.10	-51.12	891.80	-30.17	732.10	-49.90	865.90	-31.92	1191.30	-29.18	993.04	-14.68	876.54	-29.60
Cladosporium spp.	811.30	-32.23	1266.56	-0.83	1053.27	-27.92	732.10	-42.44	1336.00	-20.58	596.10	-31.27	965.88	-25.87
Macrophomina spp.	753.27	-37.08	919.32	-28.01	635.20	-56.53	683.27	-46.28	1105.40	-34.29	616.32	-28.82	785.46	-38.41
Penicillium spp.	1461.40	+22.06	1524.00	+12.32	1128.60	-22.77	1642.19	+29.10	1712.50	+1.79	1025.80	+18.46	1416.24	+10.16
Trichoderma spp.	1275.88	+6.56	1812.50	+41.91	1712.10	+17.15	1524.00	+22.16	1974.80	+17.38	1231.80	+42.25	1588.51	+24.40
Control	1197.23		1277.18		1461.40		1272.00		1682.30		865.90		1291.16	

**SVI- Seedling vigour index*

**% I or % D - (% Increase (+) or decrease (-)*

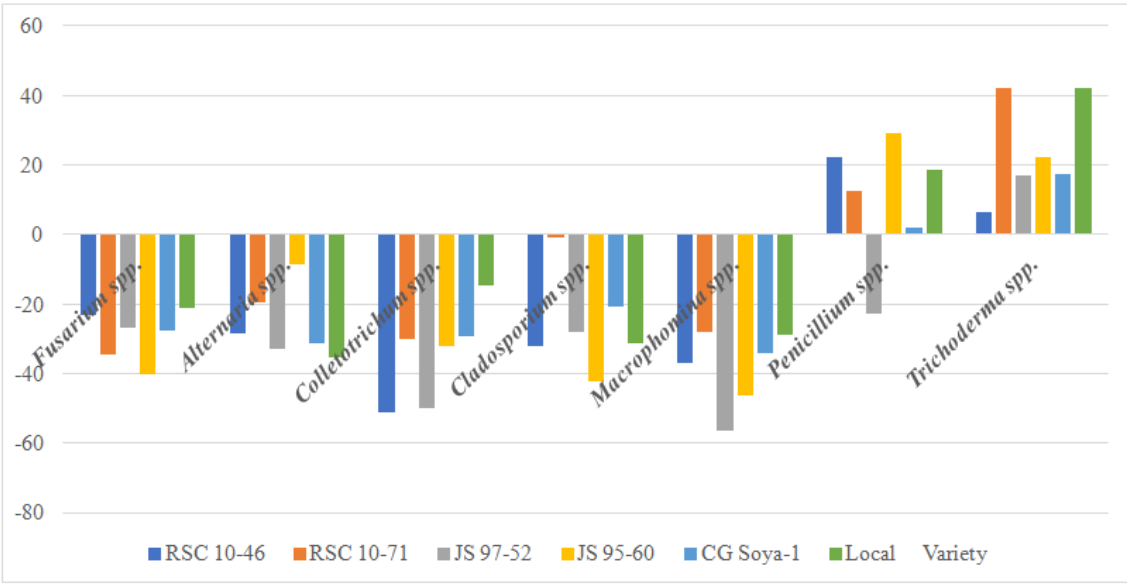

Fig. 2. Effect of seed-borne mycoflora on seedling vigour of soybean seeds (soil inoculation technique)

4. CONCLUSION

Among seven seed-borne mycoflora *viz*. *Fusarium* spp., *Alternaria spp*., *Colletotrichum spp., Cladosporium spp., Macrophomina spp., Penicillium spp*. and *Trichoderma spp.* In seed inoculation techniques maximum reduction in seedling vigour index over control was observed in *Colletotrichum* spp. (27.24%) inoculated seedlings and overall increased seedling vigour of soybean varieties was observed in *Trichoderma spp.* (26.52%) inoculated seedlings. In soil inoculation techniques maximum reduction in seedling vigour index over control was observed in *Macrophomina* spp. (38.41%) inoculated seedlings and overall increased seedling vigour of soybean varieties was observed in *Trichoderma spp.* (24.40%) inoculated seedlings.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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