DOI: 10.21088/ijps.2348.9677.12125.1

Indian Journal of Plant and Soil
Volume 12 Number 1 January - June 2025

ORIGINAL ARTICLE

Impact of Seed Mycoflora on Seed Health Status of Bottle Gourd

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HOW TO CITE THIS ARTICLE:
V. P. Prajapati, H. R. Kunvar, S. A. Rathod. Impact of Seed Mycoflora on Seed Health Status of Bottle Gourd. Ind J Plant Soil. 2025;12(1): 07-15.

ABSTRACT

The present investigation was conducted in the year 2020-2021 at the Department of Plant Protection, ASPEE College Horticulture, Navsari Agricultural University, Navsari with an aim to evaluate the impact of highly pathogenic isolates on seed germination, root length, shoot length and seedling vigour index of bottle gourd seeds var. ABG-1. Total ten seed mycoflora were isolated using agar plate method, standard blotter method and deep-freezing method viz., *Aspergillus niger, Aspergillus flavus, Alternaria alternata, Colletotrichum sp., Curvularialunata, Fusarium oxysporum, Nigrospora oryzae, Penicillium sp., Rhizoctonia sp., and Rhizopus stolonifera.* After the pathogenicity teston seeds of bottle gourd var. ABG-1 under *in vitro* (standard paper towel) and *in vivo* (pot method) condition, *Alternaria alternata, Colletotrichum* sp. *Curvularia lunata, Fusarium oxysporum* and *Rhizopus stolonifer* were evaluated to check their impact on the seed germination, shoot length, root length and seedling vigour using paper towel method. Result revealed that the seed inoculate with *Fusarium oxysporum* caused significantly lower per cent of germination (22.00%), shoot and root length (1.74cm, 4.31cm) and seedling vigour index 133.67 followed by *Colletotrichum* sp. (25.00%, 1.89cm, 4.10cm and 149.60).

KEYWORDS

• Seed mycoflora • Bottle gourd • Seedling vigour index

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Received: 17-05-2025 **Accepted:** 16-06-2025

INTRODUCTION

India is one of the major vegetables producing country in the world. India ranks second in the global vegetable production next to China. Among the various vegetable crops cultivated in India, Cucurbits are the largest group of summer vegetables grown all over the tropical countries.[11] The main cucurbits grown in India are cucumber, snack squash, watermelon, snake melon, bottle gourd, bitter gourd, pumpkin, sponge gourd, squirting cucumber, round gourd etc.[5] Amongst them, bottle gourd (Lagenaria siceraria L.) occupies a high amount in the human diet.[6] Bottle gourd is widely cultivated throughout the tropics, especially in India, Sri Lanka, Indonesia, China, and South America. In India, it is generally cultivated in the warm season and widely cultivated in all the states having an area of about 1.58 million hectares, yielding 2.6 million hectares of produce.[4] While in Gujarat, it is grown all over the districts. Among them, Ahmedabad, Vadodara, Sabarkantha, Jamnagar, Junagadh, Surat, Navsari and Valsad are the leading districts for its cultivation and production. South Gujarat alone has cultivated 5124.00 ha with a production of 90727.56 MT. Whereas, in Navsari district bottle gourd cultivated area is about 2682.00 ha with a production of 46961.82 MT in the year 2019-20.[3] Bottle gourd is a nutrient-rich vegetable, containing 96.10% water and essential nutrients per 100g of edible portion, including 0.20g protein, 2.50g carbohydrates, 0.60g fiber, 12 kcal energy, and important minerals like calcium (120 mg), magnesium (5 mg), phosphorus (10 mg), and iron (0.70 mg), along with vitamins such as vitamin C (6 mg), thiamine, riboflavin, and nicotinic acid.[9]

Despite its nutritional value, bottle gourd cultivation is threatened by a range of seed-borne fungal pathogens. These fungi adversely affect seed germination, seedling emergence, and vigour, leading to reduced plant growth and yield.[1] The development of seed-borne fungi is influenced by seed moisture, storage duration, temperature, and level of seed invasion.[5]

Fungal contamination can result in seed discoloration, poor germination, and seedling mortality, posing a major threat to successful cultivation. The seed *mycoflora associated with bottle gourd viz., Drechslera tetramera, Alternaria alternata, Fusarium moniliforme, Rhizoctonia solani, Aspergillus niger, Aspergillus flavus, Chaetomium sp., Fusarium equiseti, Fusarium solani, Curvularia lunata, Botryodiplodia theobromae, Myrothecium roridum, Sclerotium rolfsiiand Macrophomina Phaseolina* which deteriorate the quality of bottle gourd seeds therefore, it directly effects the bottle gourd cultivation.[13] Hence, it is crucial to know the impact of these mycoflora on the seed health status to ensure better crop establishment and productivity.

MATERIALS AND METHODS

1. Collection of Bottle gourd Seed Samples

The bottle gourd seeds irrespective of variety/germplasm were collected from farmer's fields, research station as well as from local markets. All collected seeds irrespective of variety/germplasm and location of collection were thoroughly mixed together in order to have a composite seed sample.

2. Detection, Isolation, Purification, Identification, and Maintenance of the Fungus Cultures

2.1 Isolation of the Fungal Culture

Isolation of external and internal seed borne mycoflora by adopting following standard International Seed Testing Association (ISTA) methods:

Agar Plate method

The water agar medium containing 2 per cent agar in distilled water was prepared and poured in volumetric flasks. The flasks were plugged with non-absorbent cotton plugs, wrapped with paper with the help of rubber band and autoclaved at 15 psi pressure for 20 min. This autoclaved medium was poured aseptically into Petri plates previously sterilized in hot air oven at 180°C for 20 minutes and allowed to solidify. Thereafter, five seeds were placed in each Petri plate and incubated at 25±2°C in BOD incubator. The incubated plates were observed under the stereoscopic microscope after 48hrs till 10 days for presence of seed-borne mycoflora.

Standard Blotter Method

Sterilized blotter paper discs were placed in sterile plastic Petri plate (155mm diameter) and moistened with sterile distilled water. The excess water was drained off from the plates.

Bottle gourd seeds were put on the plates containing the moist blotter paper. Ten seeds per plate were placed at equidistance in a circle. Four hundred seeds from composite sample were placed in the plates in four repetitions. The plates were incubated at 25 ± 2°C for seven days under alternate cycles of 12hrs light and 12hrs 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

The procedure followed for this method was almost the same as used in moist blotter paper method with the difference that in deep freezing method. The Petri plates containing bottle gourd seeds were first incubated 25 ± 2 °C for one day then after it transferred to -20°C deep freezer for 24 hrs. Deep freezed Petri plates were again incubated at 25 ± 2°C for 5 days. The Petri plates were observed, for presence of various fungi, after one week till ten days.

2.2.2 Purification of Fungal Cultures

The fungal growths of different fungi obtained on seeds were transferred on PDA Petri plates. Each fungal species isolated was further purified by hyphal tip method. Various cultures obtained were maintained on PDA slants for further study.

2.2.3 A Identification and Maintenance of the Fungal Cultures

Various seed-infecting fungi developed on bottle gourd seeds, were separately cultured on PDA Petri plates. Each fungal growth was critically observed under microscope for cultural and morphological characters. Finally, fungal characteristics observed were compared with the characteristics described in various published literature and identification was done by referring authentic relevant literature.[8,2,15] Cultures were maintained on PDA slants by sub culturing and stored at 5°C for further study.

2.3 Pathogenicity Test

For proving the Koch's postulates, the pathogens isolated from bottle gourd seeds viz., *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata, Colletotrichum* sp., *Curvularia lunata, Fusarium oxysporum, Nigrospora oryzae, Penicillium* sp., *Rhizoctonia* sp., and *Rhizopus stolonifer* were tested in *in vitro* and *in vivo* condition by adopting standard methods of pathogenicity test.

2.3.1 Pathogenicity Test *In Vitro* by Paper Towel Method

For proving pathogenicity test of various isolated fungi, healthy seeds of bottle gourd var. ABG-1 were surface sterilized with 0.1 per cent solution of mercuric chloride or sodium hypochlorite for 1 minute followed by three subsequent washings in sterilized distilled water. For artificial inoculation, moistened the seed by sterilized water was mixed thoroughly with 10 days old respective fungal culture. Four repetitions with 100 seeds were placed on wax coating paper towel and observation was taken after 8 days after sowing.

One sheet of germination paper towel was wetted by sterilized distilled water 50 seeds of respective treatment were placed on first sheet evenly. Second sheet of germination paper towel was placed on first sheet followed by wetting it carefully. Both the sheets of paper towel were rolled along with wax coated paper. The rolled papers were kept in a slanting position in plastic tray and incubated at 25 ± 2°C for 7days. At the end of incubation period, rolled towel papers were carefully opened. Germinated and un-germinated seeds were counted from each of the treatments. Emergence of seedling from the seeds was considered as successful germination. Four repetitions each of hundred seeds were maintained for each of the treatments.

2.3.2 Pathogenicity Test In Vivo by Pot Method

Apparently healthy seeds of bottle gourd var. ABG-1 were surface sterilized with 0.1 per cent mercuric chloride solution for 1 minute followed by three subsequent washings in sterilized distilled water. These seeds were soaked for 24 hrs in sterile water. They were inoculated by rolling on 10 days old actively sporulating culture of each test fungus. Then the inoculated seeds with each culture were placed in equidistance at 2-3cm depth in each pot (10 seeds/pot). The pots were watered as and when required in poly house, adopting standard techniques of pathogenicity test.

The observations on germination were recorded 8th and seedling mortality on 15th DAS.

Pre-emergence and post-emergence mortality were calculated by following formula:

$$\text{Pre-emergence mortality (\%)} = \frac{\text{No. of un-germinated seeds/pot}}{\text{No. of sown seeds/pot}} \times 100$$

$$\text{Post-emergence mortality (\%)} = \frac{\text{No. of died seedling}}{\text{No. of survived seedling}} \times 100$$

2.4 Impact of Mycoflora on Seed Health Status

2.4.1 Effect of Seed Infecting Fungi on Seed Health Status with Respect to Seed Germination and Seedling Vigour

The experiment was conducted at laboratory of Plant Pathology, Department of Plant Protection of ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari during 2020-2021 on var. ABG-1. It included six treatments in a completely randomized design with four repetitions.

Healthy bottle gourd seeds var. ABG-*1 were artificially inoculated with each of five fungal species viz., Alternaria alternata, Curvularia lunata, Colletotrichum sp., Fusarium oxysporumand Rhizopus stolonifer.* separately. For artificially inoculation, seeds moistened by sterilized distilled water were mixed thoroughly with 10 days old respective fungal culture growth were obtained at 25 ± 2°C on PDA plates.

Hundred seeds of respective treatment were placed on first sheet evenly. Second sheet of germination towel paper was placed on first sheet followed by wetting it carefully. Both sheets were rolled along with wax coated paper. The rolled papers were incubated in seed germinator at 25°C for 7 days. At the end of incubation period, rolled towel papers were carefully opened. Germinated and ungerminated seeds were counted from each of the treatments. Emergence of seedling from the seeds was considered as successful germination. After end of incubation period, these seeds were used for study of seed germination and seedling vigour index. Un-inoculated seeds served as control treatment for comparison.

Impact of seed infecting fungi on seed health status was studied in respect of seed germinability and seedling vigour from artificially inoculated seeds with fungi isolated from naturally infected bottle gourd seeds. Seedling vigour index was determined on the basis of seed germination as well as shoot and root length of seedlings. Vigour index was calculated by using following formula:

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

RESULT AND DISCUSSION

1 Collection of Bottle Gourd Seed Samples

Seed samples of different varieties of bottle gourd viz., ABG-1 and local were collected from Regional Horticulture Research Station, NAU, Navsari as well as from various markets/ village of Navsari and Valsad districts (Table 1). A composite sample of all varieties was prepared by mixing the individual variety samples and preserved in brown paper bags at P.G. Laboratory, Department of Plant Protection, ASPEE College of Horticulture and Forestry, NAU, Navsari for further investigation.

Table 1: Collection of bottle gourd seed samples

District	Research Station / Village / Market	Variety
Navsari	Regional Horticulture Research Station, NAU, Navsari	ABG-1
Navsari	Vijalpore Market	Local
Navsari	Chikhli Market	Local
Navsari	Vansda (Lakadbari village)	Local
Valsad	Dharampur (Kangavi village)	Local

2. Detection, Isolation, Purification, Identification and Maintenance of Bottle Gourd Seed Infecting Fungi

Isolation of seed-borne mycoflora from composite sample of bottle gourd seeds were carried out by agar plate method, standard blotter method and deep-freezing method by surface and without surface sterilized seeds revealed the association of ten different predominant fungi. These fungi were initially designated as isolate number 1 to 10 (Table 2). The different isolates obtained from bottle gourd seeds were purified by hyphal tip and single spore isolation technique. After purification, each of the isolate was identified on the basis of their cultural and morphological characteristics described in various authentic relevant published literature.

Table 2: Mycoflora associated with naturally infected bottle gourd seeds

Isolated No.	Fungi	Agar plate method		Standard blotter method		Deep freezing method	
		SS	US	SS	US	SS	US
1.	*Aspergillus niger*	*	*	*	*	*	*
2.	*Aspergillus flavus*	*	*	*	*	*	*
3.	*Alternaria alternata*	*	*	-	-	-	-
4.	*Colletotrichum* sp.	-	*	-	*	-	*
5.	*Curvularialunata*	*	*	-	*	-	-
6.	*Fusarium oxysporum*	*	*	*	*	-	*
7.	*Nigrospora oryzae*	-	*	*	*	-	-
8.	*Penicillium* sp.	*	*	-	-	-	*
9.	*Rhizoctonia* sp.	*	*	*	*	-	-
10.	*Rhizopus stolonifer*	*	*	*	*	-	*

*Present, – Absent, SS-Surface sterilized, US- Un-sterilized

3 Pathogenicity Test

The pathogenic nature of *Aspergillus niger, Aspergillus flavus, Alternaria alternata, Colletotrichum* sp., *Curvularialunata, Fusarium oxysporum, Nigrospora oryzae, Penicillium* sp., *Rhizoctonia* sp., *and Rhizopus stolonifer* were tested on seeds of bottle gourd var. ABG-1 under in vitro (standard paper towel) and in vivo (pot method) condition. The apparently healthy seeds of var. ABG-1 were inoculated respective fungus in individual.

1. Pathogenicity Test *In Vitro* by Paper Towel Method

The surface sterilized healthy seeds of var. ABG-1 were inoculated with 10 days old culture of each test fungus and 100 seeds for each fungus were placed on paper towel, whereas un-inoculated seeds were served as control. The data presented in Table 3, revealed that inoculation of all fungi isolated from seeds of bottle gourd has reduced germination and caused pre and post-emergence mortality as compared to un-inoculated seeds. Per cent pre-emergence mortality was found higher in the seeds inoculated with Alternaria alternata (82.00%) as compared to the rest of mycoflora fungi. Next higher pre-emergence mortality was recorded in *Fusarium oxysporum* (80.00%) followed by *Colletotrichum* sp. (78.00%), *Rhizopus stolonifer* (76.00%), *Curvularialunata* (74.00%), *Penicillium* sp. (72.20%), *Aspergillus niger* (70.00%), *Rhizoctonia* sp. (66.50%) and *Nigrospora oryzae* (60.00%). The lowest pre-emergence mortality was observed in the seed inoculated with Aspergillus flavus (58.10%), whereas pre-emergence mortality in control was observed as 20.00 per cent.

Per cent post-emergence mortality was found higher in the seeds inoculated with *Fusarium oxysporum* (65.57%) as compared to the rest. Next higher post-emergence mortality was recorded in *Alternaria alternata* (63.63%) followed by *Colletotrichum* sp. (60.00%), *Rhizopus stolonifer* (57.14%), *Curvularialunata* (50.00%), *Aspergillus niger* (40.00%), *Aspergillus flavus* (33.33%), *Rhizoctonia* sp. (29.41%) and *Penicillium* sp. (23.08%). It was the lowest in seeds inoculated with *Nigrospora oryzae* (15.00%). In control, post-emergence mortality was observed as 12.50 per cent. Reduction in germination in each treatment was caused due to rotting of seeds by inoculated fungi.

Table 3: Pathogenicity of isolated fungi on bottle gourd *in vitro*

Treat. No.	Name of Fungi	Pre-emergence mortality (%)*	Post-emergence mortality (%)*
1	*Aspergillus niger*	70.00	40.00
2	*Aspergillus flavus*	58.10	33.33
3	*Alternaria alternata*	82.00	63.63
4	*Colletotrichum* sp.	78.00	60.00
5	*Curvularialunata*	74.00	50.00
6	*Fusarium oxysporum*	80.00	65.57
7	*Nigrospore oryzae*	60.00	15.00
8	*Penicillium* sp.	72.20	23.08
9	*Rhizoctonia* sp.	66.50	29.41
10	*Rhizopus stolonifer*	76.00	57.14
11.	Control	20.00	12.50

* Average of four repetitions

3.2 Pathogenicity Test *In Vivo* by Pot Method

The surface sterilized apparently healthy seeds of var. ABG-1 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 4) that inoculation of all fungi isolated from seeds of bottle gourd 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 *Alternaria alternata* (92.50%) followed by *Fusarium oxysporum* (87.50%), whereas the highest post-emergence mortality was observed in *Fusarium oxysporum* (66.67%), which was followed by *Alternaria alternata* (64.00%) and *Colletotrichum* sp. (58.33%). The lowest pre and post-emergence mortality was observed in the seeds inoculated with *Aspergillus flavus* (55.00 and 29.41%), respectively. Reduction in germination in each treatment was caused due to rotting of seeds by inoculated fungi.

The present results are more or less similar with[10] conducted pathogenicity under in vitro condition by using seed infestation method to confirm the pathogenic nature of the fungus Fasarium oxysporum which is wilt causing *fungi in* bottle gourd.[14] who carried out *pathogenicity test of two fungi* viz., *Alternaria alternata and Fusarium oxysporum by seed inoculation and pot method by using the seeds of spinach and showed that Alternaria alternata recorded the highest pre-emergence* rot followed by *Fusarium oxysporum.* Similarly, *Fusarium oxysporum* showed the highest post-emergence rot followed by Alternaria alternata. However,[12] carried out pathogenicity test on seeds of bottle gourd under screen house *conditions showed that* seeds *inoculated with Fusarium proliferatum* and *Fusarium incarnation-equiseti* (species complex) were responsible for significant reduction in seed germination.

Table 4: Pathogenicity of isolated fungi on bottle gourd in vivo

Treat. No.	Name of Fungi	Pre-emergence mortality (%)*	Post-emergence mortality (%)*
1.	*Aspergillus niger*	72.50	40.00
2.	*Aspergillus flavus*	55.00	29.41
3.	*Alternariaalternat*a	92.50	64.00
4.	*Colletotrichum* sp.	82.50	58.33
5.	*Curvularialunata*	75.50	54.54
6.	*Fusarium xysporum*	87.50	66.67
7.	*Nigrospore oryzae*	57.50	44.44
8.	*Penicillium*sp.	70.00	50.50
9.	*Rhizoctonia* sp.	67.50	38.46
10.	*Rhizopus stolonifer*	82.00	57.14
11.	Control	12.50	00.00

*Average of four repetitions

3.4 Impact of Mycoflora on Seed Health Status

3.4.1 Impact of Seed Infecting Fungi on Seed Health Status with Respect to Seed Germination and Seedling Vigour

Inoculation of bottle gourd seeds with mycoflora viz., Alternaria alternata, Colletotrichum sp. *Curvularialunata, Fusarium oxysporumand Rhizopus stolonifer* revealed that they significantly reduced the seed germination, shoot length, root length and seedling vigourin comparison to control (Figure 1).

Seed inoculated by *Fusarium oxysporum* caused *significantly lower per cent of germination* (22.00%) followed by *Colletotrichum* sp. (25.00%), *Rhizopus stolonifer* (41.00%), *Alternaria alternata* (42.00%) and Curvularia lunata (43.00%). Overall, fungi significantly induced 53.26 to 76.09, 48.18 to 68.36 and 52.24 to 67.34 per cent reduction in seed germination, shoot length and root length over healthy seeds, respectively. The seedling vigour index (SVI) was also reduced in all the inoculated seed, by each fungus from 133.67 to 338.89 in comparison to control i.e. 1511.26 (Table 5 and Figure 2). The results in terms of shoot and root length with seedling vigour index, all the treatments showed shorter shoot length, root length and seedling vigour index as compared to control. Seed inoculated with *Alternaria alternata* recorded shoot length (2.85cm), root length (5.22cm) and *seedling vigour index* (338.89). Similarly, *Colletotrichum* sp. (1.89cm, 4.10cm and 149.60), *Curvularia lunata* (2.21cm, 3.57cm and 248.76), *Fusarium oxysporum* (1.74cm, 4.31cm and 133.67) and *Rhizopus stolonifer* (2.25cm, 5.08cm and 300.54) also recorded less shoot length, root length and seedling vigour index, respectively as compared to control (5.50cm, 10.93cm and 1511.26).

These results are more or less in agreement with[12] noted that *Fusarium proliferatum* recorded the maximum *germination inhibition* was upto 69.30 per cent followed by *Fusarium incarnation-equiseti* (60.70%) and *Colletotrichum lagenarium* recorded 55.30 per cent while seeds inoculated with the isolates *Rhizopus oryzae, Penicillium polonicum* and *Talaromyces Pinophilus* resulted decrease bottle gourd seed germination and seedling vigour.[7] observed that among the various mycoflora isolated from chilli seeds *Colletotrichum* sp. reduced 68.31 per cent seed germination followed by *Fusarium oxysporum* (65.11%) and *Alternaria alternata* (61.33%). Regarding seedling vigour index *Colletotrichum* sp. reduced the maximum seedling vigour followed by *F. oxysporum* and *A. alternata.*

Figure 1: Impact of seed infecting fungi on seed health status with respect to seed germination and seedling vigour

Table 5: Impact of seed infecting fungi on seed health status in respect to seed germination and seedling vigour

Fungi	Seed germination (%)*	Decrease in 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)
T1 – *Alternaria alternata*	42.00	54.35	2.85	48.18	5.22	52.24	338.89
T2 - *Colletotrichum* sp.	25.00	72.83	1.89	65.64	4.10	62.49	149.60
T3 - *Curvularialunata*	43.00	53.26	2.21	59.82	3.57	67.34	248.76
T4 - *Fusarium oxysporum*	22.00	76.09	1.74	68.36	4.31	60.57	133.67
T5 - *Rhizopus stolonifer*	41.00	55.43	2.25	59.09	5.08	53.52	300.54
T6 - Control	92.00	–	5.50	–	10.93	–	1511.26
SEm ±	0.85	–	0.05	–	0.07	–	7.17
CD at 5%	2.52	–	0.14	–	0.22	–	20.92
CV %	3.85	–	3.35	–	3.55	–	4.27

*Average of four repetitions

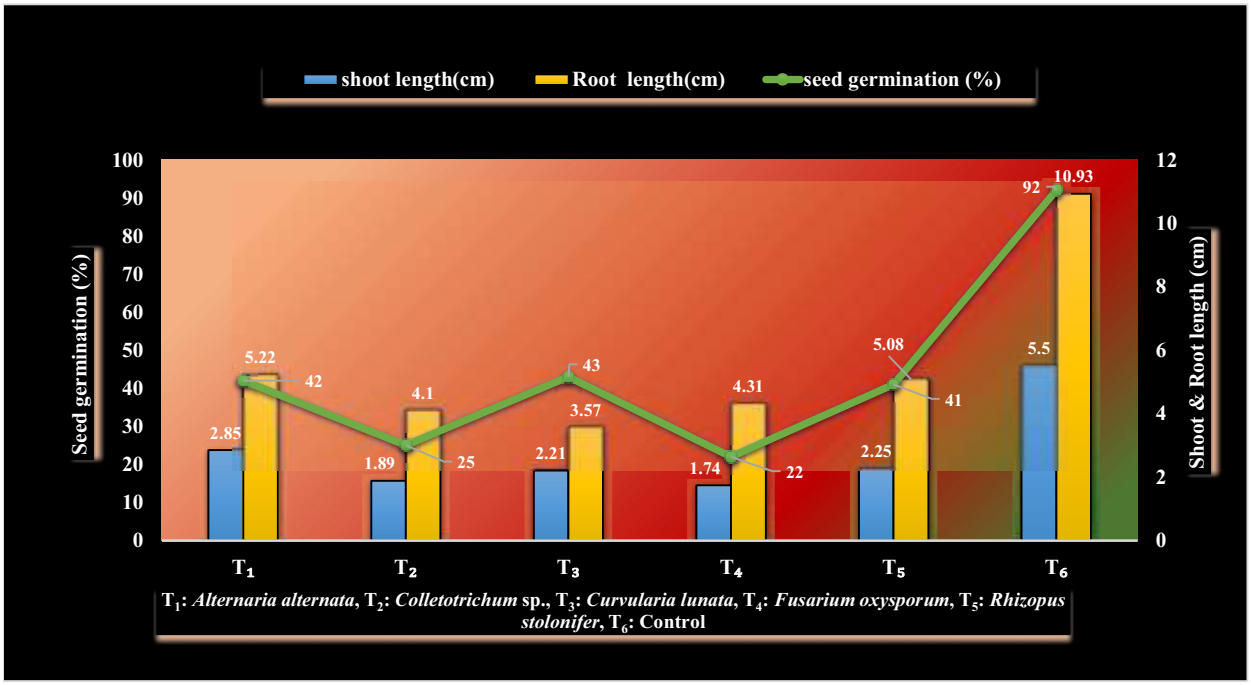

Figure 2: Impact of seed infecting fungi on seed health status with respect to seed germination and seedling vigour

CONCLUSION

The seeds act as a passive carrier of pathogens that are transmitted when seeds germinated under suitable environmental conditions and held progressive reduction in the concomitant loss of viability due to seed borne fungal spores. Fungi associated with seeds act as the principle devastating organisms causing adverse impact on seed germination and seedling emergence, seedling vigour, plant growth and ultimately on the quality and quantity of the production. The present investigation isolated ten different mycoflora associated with bottle gourd seeds and recorded that seed inoculate with *Fusarium oxysporum* caused significantly lower per cent of *germination* (22.00%), shoot and root length (1.74cm, 4.31cm) and *seedling vigour index* 133.67 followed by *Colletotrichum* sp. (25.00%, 1.89cm, 4.10cm and 149.60).

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