

Detrimental effects of seed-borne microflora on seed germination and seedling vigour of Cabbage

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Seed-borne microflora significantly threatens cabbage (*Brassica oleracea* L. var. *capitata*) production by reducing germination and seedling quality. This study investigated the pathogenicity and impact of isolated microflora on the seed health status of cabbage. Ten microorganisms including *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* sp., *Curvularia* sp., *Fusarium* sp., *Macrophomina* sp., *Alternaria* sp., *Penicillium* sp., *Helminthosporium* sp., and the bacterium *Xanthomonas* sp. were tested using *in vitro* (rolled paper towel) and *in vivo* (pot method) assays. Results indicated that all tested microflora negatively impacted seed health. In the *in vitro* pathogenicity test, *Rhizopus* sp. caused 100 per cent pre-emergence mortality. *A. niger* and *Curvularia* sp. also showed high pathogenicity, with *A. niger* recording the highest post-emergence mortality (46.45%). *In vivo* results mirrored these findings, where *Curvularia* sp. and *A. niger* caused pre-emergence mortality of 79.89 per cent and 75.25 per cent, respectively. Further evaluation of five dominant fungi revealed significant reductions in growth parameters compared to the control. *A. niger* was the most detrimental, reducing seed germination to 25.75 per cent (a 53.60% decrease over healthy seeds) and resulting in a Seedling Vigour Index (SVI) of only 24.97, compared to the control SVI of 508.49. Overall, fungal inoculation led to up to 95.88 per cent reduction in root length and 76.59 per cent in shoot length. These findings underscore the critical need for effective seed health management to ensure successful cabbage cultivation.

Keywords : Cabbage, pathogenicity, seed germination, seed health, seed mycoflora, seedling vigour index

INTRODUCTION

Cabbage (*Brassica oleracea* L. var. *capitata*) is a widely enjoyed temperate vegetable and a prominent member of the Brassicaceae family which is a vast group of flowering plants containing roughly 3709 species across 360 genera globally. Because of its versatility, cabbage holds a significant place in international culinary traditions.

It is prepared in numerous ways viz., shredded for fresh salads, boiled, stir-fried or fermented for

long-term use. Beyond human consumption, cabbage serves as a common feed for poultry and livestock in developed nations. Its low cost and consistent presence in local markets make it a fundamental dietary staple for people everywhere (Šamec *et al.* 2019).

Valued at USD 5.21 million in 2025, the global cabbage market is projected to reach USD 6.79 million by 2033, expanding at a Compound Annual Growth Rate (CAGR) of 3.37 per cent driven by rising consumption across various regions and sectors. As one of the top five vegetable crops worldwide, cabbage is cultivated in over 90 countries with global production surpassing 71 million metric tons in 2024. China dominates the

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industry, accounting for more than 47 per cent of the total output at over 33 million metric tons, followed by India as the second largest producer at approximately 9 million metric tons, alongside other major contributors like Russia, South Korea and Japan (Anon., 2026a). In India, West Bengal ranks first in cabbage production in 2024-25, with a total production of 2385.95 million tons. Odisha ranks second with a total production of 1305.59 million tons. Madhya Pradesh ranked third, producing approximately 1102.34 million tons. Gujarat ranks fourth with an annual production of 870.81 million tons, covering an area of 37.38 thousand hectare and productivity of 23.30 million tons per hectare (Anon., 2026b).

The presence of pathogens in cabbage seeds can significantly diminish agricultural productivity, resulting in substantial yield losses and inferior seedling development. This lack of quality leads to diminished market prices, subpar germination rates and difficulty in establishing healthy crops in the field (Akaranuchat *et al.* 2008). Seeds are fundamental for successful crop production but they frequently act as vectors for various plant pathogens. These seed-borne diseases stemming from bacterial, fungal and viral sources can inhabit the seed both internally and externally, allowing infections to persist into subsequent growing seasons. This transmission poses a major risk to overall agricultural yields, with fungal contamination representing the most significant threat to crop health (Amza, 2018). Agricultural damage resulting from seed microflora, which encompasses various bacteria and fungi can manifest at multiple stages, including initial seed development, during storage and throughout the germination process (Mehrotra and Aggarwal, 2003). Seeds are frequently colonized by diverse microflora that can inflict severe damage, ranging from seed abortion and shrunken or reduced seed size to more destructive conditions like rot, necrosis, discoloration and sclerotization. Beyond physical deformities, these pathogens can trigger physiological alterations and lead to a partial or total loss of germination viability. Once these infected seeds sprout, the pathogens can migrate to seedlings, vegetative buds or mature cabbage heads, eventually causing total plant failure through the systemic deterioration of essential tissues (Amza, 2018).

According to Dorna *et al.* (2010), inoculating seeds with *Alternaria brassicicola* does not entirely prevent germination, but it drastically reduces overall germination rates while increasing the occurrence of infected seedlings and seed mortality. Their research on priming methods indicated that heavily infected seed lots respond poorly to standard vigour tests, suggesting that the fungus damages the embryo's metabolic potential during the early imbibition phase. Similarly, Dhekle (2016) conducted an extensive survey of fungal diversity on leafy vegetables in Maharashtra. He isolated 15 species, with *Aspergillus nigervan* Tieghem, *Aspergillus flavus* Link, *Fusarium oxysporum* Schltdl. and *Curvularia lunata* (Wakker) Boedijn being the most dominant. These fungi were found to be seed to seedling transmissible, meaning they not only reduced germination but also caused root necrosis in the surviving seedlings, significantly lowering the SVI. Meena (2020) carried out a standard blotter test on mustard seeds and reported that *A. niger*, *C. lunata*, *F. oxysporum*, *A. brassicicola*, *Alternaria alternata* (Fries) Keissler and *Alternaria brassicae* (Berk.) Sacc. suppress seed germination and drive-up mortality rates both before and after seedling emergence compared to uninfected controls. Hakalová *et al.* (2022) highlighted that even low levels of seed contamination can lead to systemic infection in cabbage seedlings. Their study demonstrated that the bacterium *Xanthomonas* ssp. colonizes the seed coat, endosperm or embryo, significantly reducing seedling vigour before typical symptoms (V-shaped lesions) even appear.

MATERIALS & METHODS

Pathogenicity Test

To know the pathogenicity of the microflora isolated from cabbage seeds obtained from Regional Horticultural Research Station, Navsari Agricultural University, Navsari the seed-borne microflora viz., *A. niger*, *A. flavus*, *Alternaria* sp., *Macrophomina* sp., *Curvularia* sp., *Fusarium* sp., *Penicillium* sp., *Helminthosporium* sp., *Rhizopus* sp. and *Xanthomonas* sp. were tested *in vitro* and *in vivo* conditions by adopting standard methods of pathogenicity test at Department of Plant

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Pathogenicity test in vitro by rolled papertowel method

To verify the pathogenicity of isolated microflora, healthy cabbage seeds underwent surface sterilization using a 0.1 per cent mercuric chloride solution for one minute, followed by three rinses in sterile distilled water. For fungal inoculation, seeds were pre-soaked for 24 hr and then thoroughly integrated with 10 day old cultures. To assess bacterial pathogenicity, a suspension was created by adding 10 ml of distilled water to 48h old nutrient agar slants, where bacterial cells were carefully suspended into the liquid using an inoculating needle by gentle scraping from culture plate (D'Souza, 2011). To evaluate germination, 100 seeds per treatment were divided into four replicates of 25 and arranged evenly between two distilled water-moistened paper towels backed by wax coated sheets. These assemblies were rolled

$$\text{Post-emergence mortality} = \frac{\text{No. of died seedlings}}{\text{No. of Survived seedlings}} \times 100$$

$$\text{Pre-emergence mortality} = \frac{\text{No. of ungerminated seeds}}{\text{No. of sown seeds}} \times 100$$

Pathogenicity test in vivo by pot method

Healthy cabbage seeds were first surface-sterilized using a 0.1 per cent mercuric chloride solution for one minute, followed by three rinses in sterile distilled water and a 24 hr soak. To inoculate the seeds with fungal microflora, they were rolled directly onto 10 day old actively sporulating cultures. For bacterial pathogenicity testing, a suspension was prepared by adding 10 ml of distilled water to 2 day old Nutrient Agar (NA) slants and gently scraping the growth with an inoculating needle to ensure the cells were fully suspended before seed treatment (D'Souza, 2011). Following inoculation, 10 seeds were sown

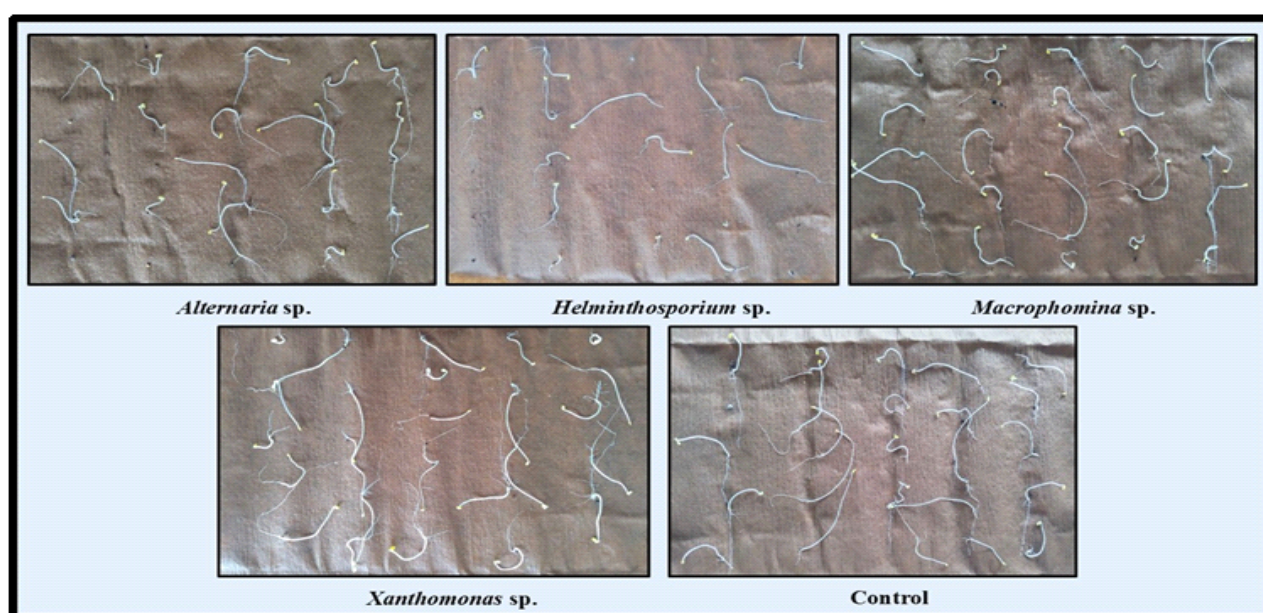


Fig.1: Pathogenicity tests of fungal isolates against cabbage seeds. Top- Rolled Paper Towel method; Bottom- Pot method

and incubated at $25 \pm 2^\circ\text{C}$ in a slanted position within plastic trays for seven days. Following incubation, the towels were opened to quantify successful germination and to record the number of ungerminated seeds.

Pre-emergence and post-emergence mortality were calculated in both methods by the following formula (Singh *et al.* 2022).

in each pot at an equidistant spacing and a depth of 2–3 cm. These pots were maintained in a polyhouse and watered as needed according to standard pathogenicity testing protocols (Fig. 2). To assess the impact of the cultures, germination rates were documented 8 day after sowing (DAS), while seedling mortality was recorded 15 DAS.

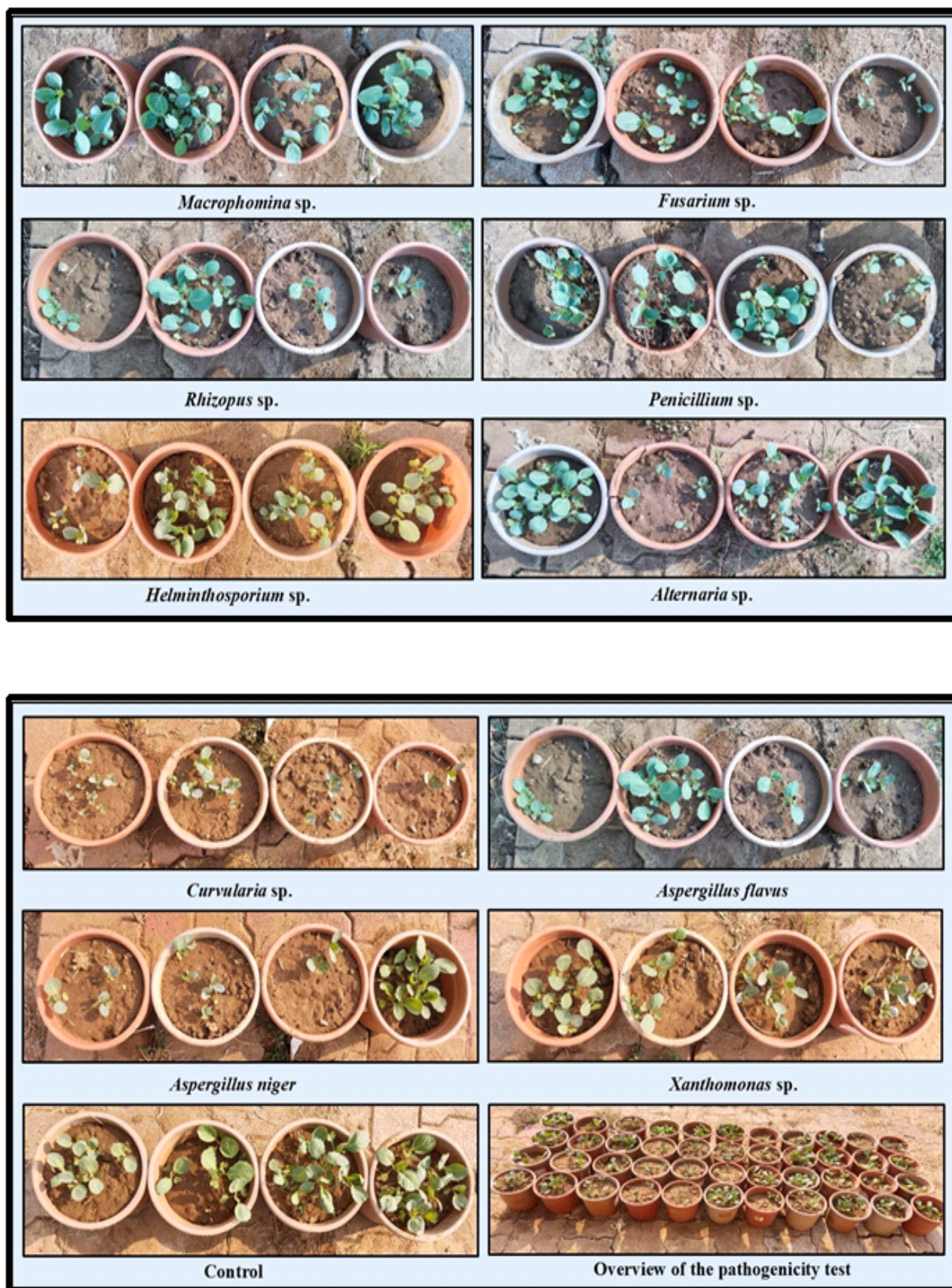


Fig.2: Pathogenicity test *in vivo* by pot method

Pre-emergence and post-emergence mortality were calculated in both methods by the following formula (Singh *et al.* 2022).

$$\text{Pre-emergence mortality} = \frac{\text{No. of ungerminated seeds}}{\text{No. of sown seeds}} \times 100$$

$$\text{Post-emergence mortality} = \frac{\text{No. of died seedlings}}{\text{No. of Survived seedlings}} \times 100$$

Impact of Micro flora on Seed Health Status of Cabbage

Healthy cabbage seeds were artificially inoculated with each of the frequently occurring five fungal species viz., *A. niger*, *A. flavus*, *Curvularia* sp., *Fusarium* sp. and *Penicillium* sp., separately. To evaluate the impact of seed-infecting microflora on seed health, five dominant fungal species were selected based on comprehensive *in vitro* and *in vivo* pathogenicity testing. *Rhizopus* sp. was not taken as treatment in this study because pre-emergence mortality of the fungus was found to be cent per cent *in vitro*, therefore it is impossible to measure root length, shoot length and seedling vigour index to *Rhizopus* sp. infected seeds *in vitro*.

Artificial inoculation was performed by mixing distilled water moistened cabbage seeds with 10 day old fungal cultures grown on PDA plates at $25 \pm 2^\circ\text{C}$. For each treatment, four replicates of 25 seeds were placed between two layers of moistened paper towels, rolled and incubated in a seed germinator at 25°C for seven days, while uninoculated seeds served as a control. Following incubation, successful germination was quantified by the emergence of seedlings. These results, combined with measurements of shoot and root lengths, were used to calculate the seedling vigour index, thereby characterizing the influence of the isolated microflora on overall seed germinability and growth potential.

The vigour index was calculated by the following formula (Mallesh *et al.*, 2008):

$$\text{Seed Germination (\%)} = \frac{\text{No. of Germinated seeds}}{\text{Total No. of Seeds}} \times 100$$

Vigour index (VI) = Per cent seed germination x (mean of root length + mean of shoot length)

RESULTS & DISCUSSION

Pathogenicity Test

The pathogenic nature of *A. niger*, *A. flavus*, *Rhizopus* sp., *Penicillium* sp., *Alternaria* sp., *Curvularia* sp., *Macrophomina* sp., *Helminthosporium* sp., *Fusarium* sp. and *Xanthomonas* sp. were tested on cabbage seeds under *in vitro* (rolled paper towel method) and *in vivo* (pot method) conditions. The healthy seeds of cabbage were inoculated with the respective microflora individually.

Pathogenicity test in vitro by rolled paper towel method

To prepare the samples, surface-sterilized healthy cabbage seeds were inoculated with either 10 day old fungal cultures or 2 day old bacterial cultures. For each specific microflora treatment, 100 seeds were tested across four replicates of 25 seeds each using the-rolled paper towel method, with uninoculated seeds maintained as a control group for comparison. The data presented in table 1 revealed that inoculation of all microflora isolated from cabbage seeds reduced germination and caused pre-emergence and post-emergence-mortality compared to un-inoculated seeds. Percent pre-emergence mortality was found higher in the seeds inoculated with the *Rhizopus* sp. (100%) as compared to the rest of the isolated microflora. Next higher pre-emergence mortality was recorded in *A. niger* (80.25%) followed by *Curvularia* sp. (76.34%), *Fusarium* sp. (68.15%), *A. flavus* (54.87%), *Penicillium* sp. (50.22%), *Helminthosporium* sp. (38.00%), *Alternaria* sp. (20.57%), *Xanthomonas* sp. (18.22%) and *Macrophomina* sp. (15.67%). The lowest pre-emergence mortality among inoculated seeds was observed in the seeds inoculated with *Macrophomina* sp. (15.67%). Whereas, the pre-emergence mortality in control was 10.00 per cent. Percent post-emergence mortality was found higher in the seeds inoculated with *A. niger* (46.45%) as compared to the rest. Next higher post-emergence mortality was recorded in

Table 1: Pathogenicity of isolated microflora on cabbage seeds *in vitro*

Treatment No.	Name of microflora	Pre-emergence mortality (%) [#]	Post-emergence mortality (%) [#]
1	<i>Aspergillus niger</i>	80.25	46.45
2	<i>Aspergillus flavus</i>	54.87	33.33
3	<i>Rhizopus</i> sp.	100.00	00.00
4	<i>Penicillium</i> sp.	50.22	21.42
5	<i>Alternaria</i> sp.	20.57	12.53
6	<i>Curvularia</i> sp.	76.34	42.62
7	<i>Fusarium</i> sp.	68.15	40.77
8	<i>Helminthosporium</i> sp.	38.00	27.56
9	<i>Macrophomina</i> sp.	15.67	6.69
10	<i>Xanthomonas</i> sp.	18.22	7.97
11	Control	10.00	5.38

[#]Average off our repetitions

Curvularia sp. (42.62%) followed by *Fusarium* sp. (40.77%), *A. flavus* (33.33%), *Helminthosporium* sp. (27.56%), *Penicillium* sp. (21.42%), *Alternaria* sp. (12.53%), *Xanthomonas* sp. (7.97%) and *Macrophomina* sp. (6.69%). In control, it was 5.38 per cent. All the isolated pathogens were found to reduce germination percentage on cabbage seeds and this was caused due to rotting of seeds by inoculated microflora (Table 1, Fig.1).

Pathogenicity test in vivo by pot method

Healthy cabbage seeds were surface sterilized and inoculated with either 10 day old fungal or 2 day old bacterial cultures. For each treatment, 10 seeds were sown in plastic pots containing sterile soil, with uninoculated seeds serving as the control group. As shown in (Table 2), all isolated microflora negatively impacted seed

health by reducing germination rates and inducing both pre and post-emergence mortality relative to the control. The highest pre-emergence mortality was recorded in seeds inoculated with *Curvularia* sp. (79.89%) followed by *A. niger* (75.25%) and *Rhizopus* sp. (67.00%) whereas the highest post-emergence mortality was observed in *Rhizopus* sp. (45.50%) which was followed by *A. niger* (42.79%) and *Curvularia* sp. (40.34%). The inoculation with *Macrophomina* sp. resulted in the minimum recorded levels of pre-emergence and post-emergence mortality, at 12.89% and 5.80% respectively. Reduction in germination in each treatment was caused due to rotting of seeds by inoculated microflora (Table 2, Fig.2)

Similar findings were shown by Rathod (2023), who carried out a pathogenicity test *in vivo* using

Table 2: Pathogenicity of isolated microflora on cabbage seeds *in vivo*

Treatment No.	Name of microflora	Pre-emergence mortality (%) [#]	Post-emergence mortality (%) [#]
1	<i>Aspergillus niger</i>	75.25	42.79
2	<i>Aspergillus flavus</i>	40.30	12.50
3	<i>Rhizopus</i> sp.	67.00	45.50
4	<i>Penicillium</i> sp.	40.41	17.52
5	<i>Alternaria</i> sp.	15.00	10.56
6	<i>Curvularia</i> sp.	79.89	40.34
7	<i>Helminthosporium</i> sp.	22.50	17.14
8	<i>Fusarium</i> sp.	37.50	18.60
9	<i>Macrophomina</i> sp.	12.89	5.80
10	<i>Xanthomonas</i> sp.	15.5	8.75
11	Control	5.50	0.00

[#]Average of four repetitions**Table 3 :** Impact of seed infecting microflora on seed health status concerning seed germination and seedling vigour

Treatments	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)
T ₁ - <i>Aspergillus niger</i>	25.75	53.60	0.72	76.59	0.25	95.88	24.97
T ₂ - <i>Aspergillus flavus</i>	52.70	5.03	2.24	27.59	5.75	5.24	421.30
T ₃ - <i>Fusarium</i> sp.	40.62	26.80	2.12	31.40	5.22	13.94	298.49
T ₄ - <i>Curvularia</i> sp.	42.50	23.42	2.21	28.66	5.47	9.77	326.72
T ₅ - <i>Penicillium</i> sp.	52.12	6.08	2.54	17.75	5.54	8.65	421.49
T ₆ - Control	55.50	---	3.09	---	6.06	---	508.49
SEm±	0.61		0.04		0.10		6.20
CD at5%	1.83		0.13		0.31		18.57
CV%	2.72		4.30		4.50		3.71

[#]Average of four repetitions

the pot method. The results revealed that among the ten different seed mycoflora of yardlong bean, the highest pre-emergence mortality was recorded in seeds inoculated with *Curvularia* sp. (72.50%) while Aswani (2022) performed the same pathogenicity test on the seed mycoflora of okra. The results showed that the highest post-emergence mortality was observed in *R. stolonifer* (66.67%) infected okra seeds.

In case of *in vitro* conditions, similar results were found by Mahesh (2024), who carried out a pathogenicity test using the rolled paper towel method. He discovered that percent pre-emergence mortality was found higher in the seeds inoculated with *A. niger* (43.00%) as compared to the rest of the onion seed mycoflora. Hussain *et al.* (2013) found that *F. moniliforme* had 50.20 per cent pathogenicity on maize seeds and 6.55 per cent on seedlings, whilst *A. niger* had 62.87 per cent on seeds and 11.24 per cent on seedlings. D'Souza (2011) reported that the inhibition of germination due to *X. campestris* pv. *campestris* was 13.64 per cent in cabbage and mustard seeds.

Impact of microflora on seed health status of cabbage

Cabbage seeds inoculated with five dominant fungi viz., *A. niger*, *A. flavus*, *Fusarium* sp., *Curvularia* sp. and *Penicillium* sp. based on their pathogenic nature, significantly reduced the seed germination, shoot length, root length and seedling vigour in comparison to the control (Fig. 3).

Seeds inoculated with *A. niger* significantly reduced the seed germination (25.75%) followed by *Fusarium* sp. (40.62%), *Curvularia* sp. (42.50%), *A. flavus* (52.70%) and *Penicillium* sp. (52.12%) over control (55.50%). Overall, fungi significantly induced 5.03 percent to 53.60 per cent, 17.75 percent to 76.59 percent, 5.24 per cent to 95.88 percent reduction in seeds germination, shoot length and root length over control, respectively. The seedling vigour index was also reduce in all the inoculated seed, by each fungus from 24.97 to 421.49 over control i.e., 508.49 (Table 3 & Fig. 4).

All the treatment showed shorter shoot length, root length and vigour index as compared to control. Seeds inoculated with *A. niger* recorded shoot length (0.72 cm), root length (0.25 cm) and vigour index (24.97). Similarly, *Fusarium* sp. (2.12 cm, 5.22 cm, 298.49), *Curvularia* sp. (2.21 cm, 5.47 cm, 326.72), *A. flavus* (2.24 cm, 5.75 cm, 421.30) and *Penicillium* sp. (2.54 cm, 5.54 cm, 421.49) also recorded lower shoot length, root length and vigour index, respectively last compared to control (3.09 cm, 6.06 cm, 508.49).

These results are more or less in conformity with Tambe *et al.* (2021), who found that the lowest seed germination and seedling vigour index in onion seeds was noticed in case of seeds inoculated with *A. niger* that is 77.33 per cent and 541.33, respectively. Pawar (2010) observed that among five dominant seed borne fungi isolated from soybean seeds *F. oxysporum* showed minimum per cent seed germination, 29.00 percent as against 83.00 percent in the control and minimum seedling vigour index, 310 as against 2296 in the control.

Statistical analysis was done using Completely Randomized Design (CRD) through opstat software

CONCLUSION

The research confirms that seed-borne microflora, particularly fungal pathogens, pose a substantial risk to the successful cultivation of cabbage by compromising both seed viability and subsequent plant development. The study demonstrates that these microorganisms significantly impair the health status of cabbage seeds, leading to high rates of pre- and post-emergence mortality. Furthermore, the presence of these pathogens results in a marked deterioration of essential growth parameters, including germination percentage and seedling vigour, effectively reducing the overall metabolic potential of the crop. These findings highlight the detrimental impact of seed-infecting fungi on initial crop establishment and underscore the imperative for robust seed health management protocols to safeguard agricultural productivity and ensure healthy crop yields.

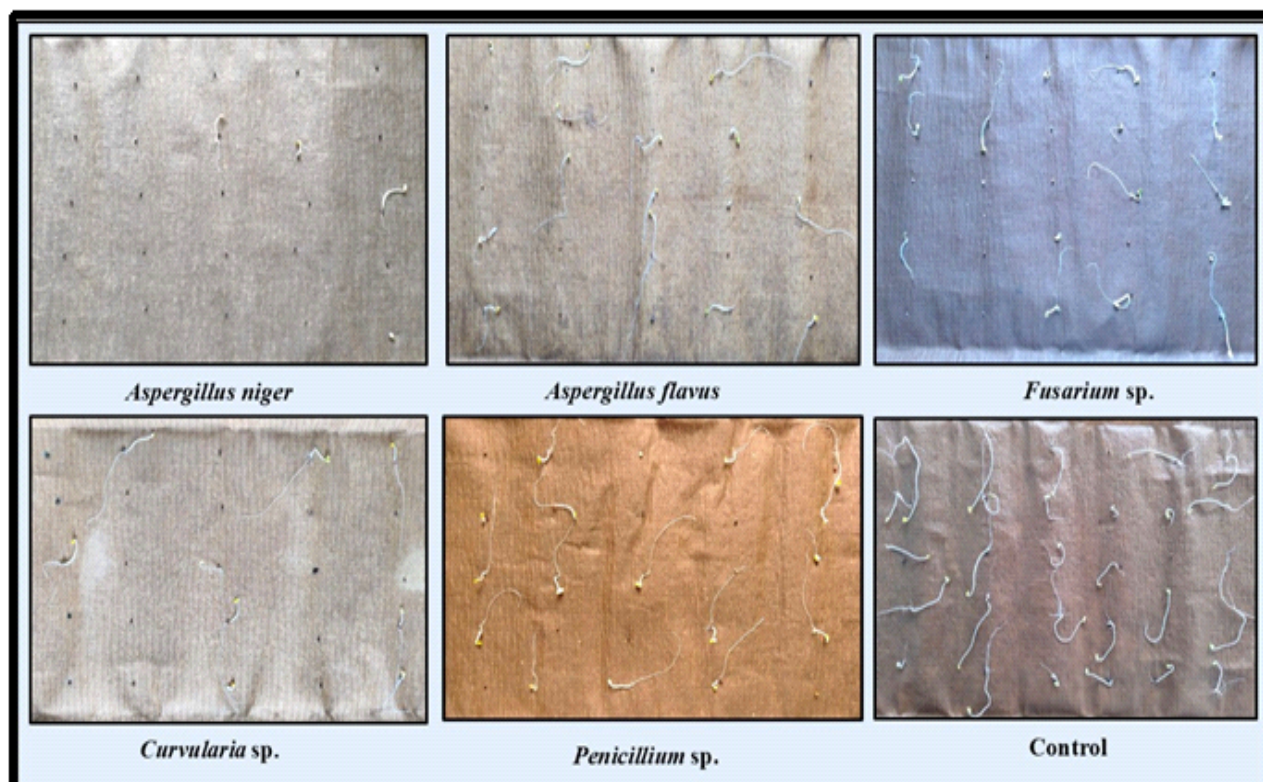


Fig. 3: Impact of seed infecting microflora on seed health status concerning seed germination and seedling vigour

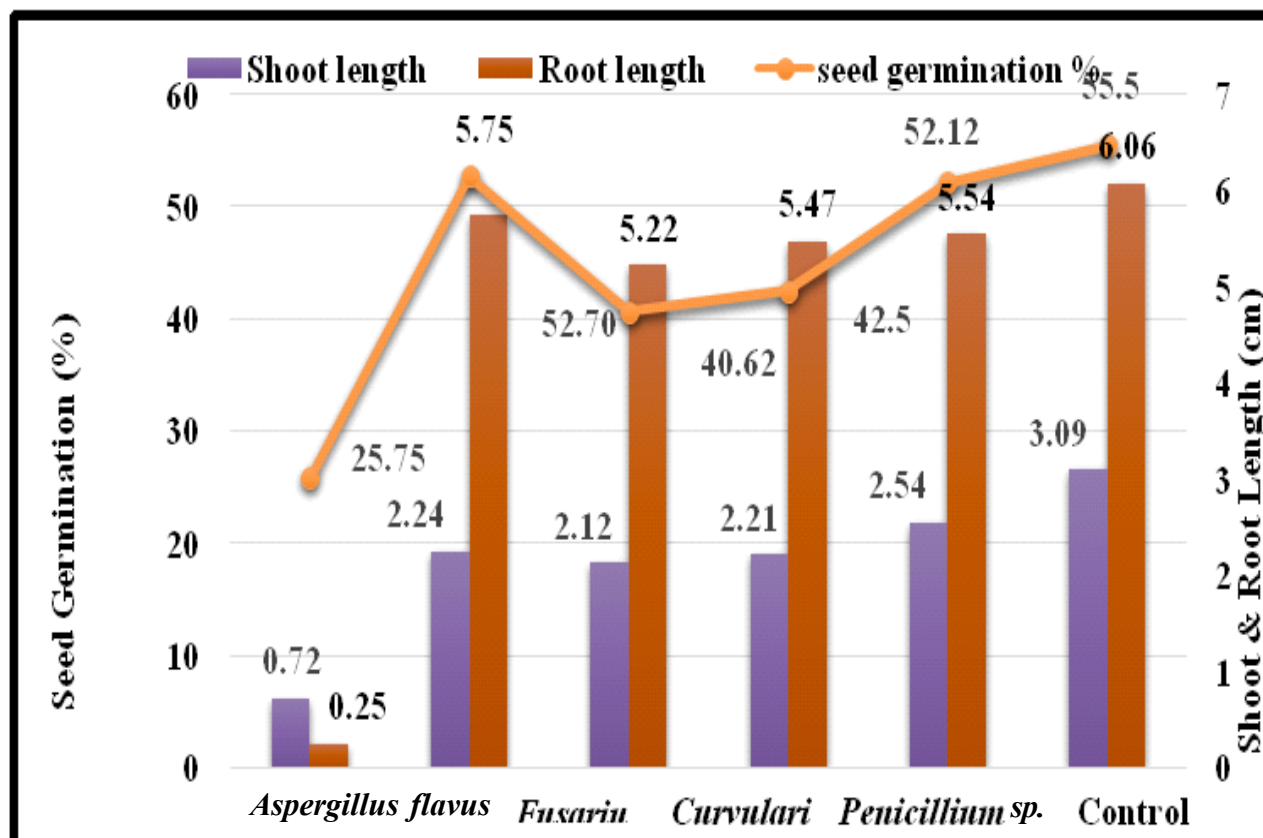


Fig. 4: Impact of seed infecting microflora on seed health status concerning seed germination and seedling vigour

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DECLARATION

Conflict of Interest. Authors declare no conflict of interest

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