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Effect of culture filtrate of seed mycoflora on seed health in respect to seed germination, seedling length and seedling vigour in fennel cultivars

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Abstract
An experiment was carried out to investigate the effect of culture filtrate of seed mycoflora on seed health regarding seed germination, seedling length and seedling vigour in fennel cultivars. Five different fennel varieties *viz*., GF 2, GF 11, GF 12, AF 1 and AF 2 were used. The seed germination, seedling length and seedling vigour index-I (SVI-I) were drastically reduced by culture filtrate of all isolated seed mycoflora in all five fennel cultivars. Seeds inoculated with culture filtrate of *M. phaseolina* showed the lowest germination, seedling length and seedling vigour index-I in all cultivars.

Keywords: Seed mycoflora, fennel, cultivar, *Macrophomina phaseolina*

Introduction
Fennel (*Foeniculum vulgare* Mill.) is a medicinal plant belonging to the *Umbelliferae* (*Apiaceae*) family. It is called *Saunf* in Hindi and *Variyali* in Gujarati. It originated in the Southern Mediterranean region and throughout the Northern, Eastern and Western hemispheres, specifically in Asia, North America and Europe. India is the world's largest fennel-producing country followed by China, Bulgaria, Iran, Mexico, Syria and Egypt. In India, fennel is mostly grown in the northern region and the important fennel-producing states are Gujarat, Rajasthan, Madhya Pradesh, Haryana and Uttar Pradesh. In India, it is grown in an area of 75,000 ha with a production of 1,28,000 MT during 2019-20 (Anonymous, 2020a) [3]. Gujarat is the largest fennel-producing state in India followed by Rajasthan. In Gujarat, it is mainly cultivated in Surendranagar, Mehsana, Banaskantha, Patan, Gandhinagar, Aravalli, Kutch, Kheda and Anand districts. It is cultivated in an area of 52,802 ha with a production of 1,09,026 MT during 2019-20 in Gujarat (Anonymous, 2020b) [4].

A series of studies shows that *F. vulgare* effectively controls numerous infections disorders of bacterial, fungal, viral, mycobacterium and protozoal origin. It has antioxidant, antitumor, chemopreventive, cytoprotective, hepatoprotective, hypoglycaemic and oestrogenic activities. Some research stated that *F. vulgare* has a special kind of memory-enhancing effect and can reduce stress. Because of its valuable nutritional makeup, including the inclusion of vital fatty acids, a diet rich in fennel could provide beneficial health effects (Dwivedi *et al*., 2008) [5]. Singh *et al.* (1990) [11] found that anethole and fenchone are the most prominent. components found in fennel oil. The pale yellow coloured essential volatile oil present in fennel seeds ranges from 1.4 to 4.0 per cent. In addition, fennel oil possesses some valuable pharmacological properties such as nematicidal, antimicrobial and detoxifying enzyme-inducing activities. Therefore, it is used in soaps and various medicinal preparations in pharmaceutical industries and as a flavoring agent. The residue left after the distillation of essential oil from the seed is used as feed for cattle as it contains about 20 per cent protein, 18 per cent fat and 18 to 20 per cent crude fiber (Lal and Sen, 1971; Nichita *et al*., 1981) [8, 10].

Seeds are an especially effective carrier for spreading seed-borne diseases. A seed-borne pathogen growing externally, internally or associated with the seed as a contaminant might cause; seed abortion, rot, necrosis, reduction or elimination of seed germination capacity and seedling vigour. As a result, seedling damage causes plant disease development at later stages of its growth. The most predominant fungal genera encountered on fennel seeds were *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp. and *Fusarium* spp. (Khanzada *et al*., 2002) [7]. The contaminated seeds do not germinate or seedlings and plants grown in the field from infected seeds may avoid an early infection but frequently contract it later in the growth process.

In addition, diseases can travel far and wide, and seeds carrying various pathogens can spread the infection to uninfected fields (Fakir *et al*., 2001) [6].

Materials and Methods

Total five fennel cultivars *viz*., GF 2, GF 11, GF 12, AF 1 and AF 2 and seven seed mycoflora isolated from these fennel cultivars namely, *Alternaria alternata, Macrophomina phaseolina, Fusarium oxysporum, Curvularia lunata, Aspergillus niger, A. flavus* and *A. fumigatus* were used for the study.

Seed Inoculation with Seed Mycoflora

Fennel seeds of five cultivars were artificially inoculated with each of the seed mycoflora separately. Seeds moistened with sterilized water were thoroughly mixed with 10 days old culture of respective fungal cultures obtained on PDA at 25 ± 2 °C. Such treated seeds were kept in Petri plates overnight at 25±2 °C and then these seeds were used for seed germination, seedling vigour index study and assessment of seedling discoloration.

Effect on Seed Germination

The effect of seed mycoflora on seed germination was tested by the top of the paper method. Germination paper was placed in a Petri plate and wetted with sterilized distilled water. Twenty-five seeds of respective fennel cultivars were inoculated with respective seed mycoflora and placed on the germination paper. The Petri plates were incubated in a seed germinator at 20 °C for 14 days. At the end of incubation, germinated and ungerminated seeds were counted treatment-wise and variety-wise. Healthy seeds without inoculation of seed mycoflora were considered as control. Four repetitions of each of 100 seeds were maintained for each of the treatments. The seedling lengths of germinated seeds were recorded.

Effect on Seedling Vigour Index-I (SVI-I)

Seedling Vigour Index-I (SVI-I) was calculated on the basis of seed germination and seedling length (root length and shoot length) after 14 days of incubation described under sub-heading 3.3.2 by using the formula given by Abdul-Baki and Anderson (1973) [1].

$$\text{SVI} - \text{I} = \text{Seedling length} \times \text{Seed germination (\%)}$$

Results and Discussion

Effect of Seed Mycoflora on Seed Germination

The data on seed germination as influenced by seed mycoflora revealed significant differences (Table 1). When compared to the control treatment, all of the tested fungi had substantial inhibitory effects on seed germination of fennel cultivars and reduced seed germination. Among the five cultivars, GF 2 (49.22%) had the lowest seed germination percentage followed by GF 11 (63.86%) and AF 1 (66.12%). The AF 2 cultivar had the highest seed germination (73.37%) followed by the GF 12 (70.57%). The maximum inhibitory effect on seed germination was observed in seeds inoculated with culture filtrate of *M. phaseolina* (38.49%) followed by *A. niger* (60.26%) which was found statistically at par with *A. flavus* (61.58%). *Curvularia lunata* had the least inhibitory effect on seed germination (72.48%) as compared to control (87.56%). Results revealed that the germination of fennel seeds in each cultivar was reduced due to the rotting of seeds by inoculated fungi. A somewhat similar result was obtained by Al-Zubaide *et al.* (2014) [2], who found that *Fusarium lateritium, F. solani* and *Rhizoctonia* sp. caused significant effects on decreasing germination percentages of black cumin seeds from 32.40 per cent, 31.94 per cent and 7.87 per cent, respectively compared with the control (83.33%). Zhang *et al.* (2020) [13] also recorded that *A. alternata* decreased the germination rate of carrot seeds up to 28.7 per cent.

Effect of seed mycoflora on seedling length

The top of the paper method was used for the assessment of seedling length of five distinct fennel cultivars as influenced by seed mycoflora. The data presented in Table 2 revealed that all the tested fungi significantly reduced seedling length as compared to the control. The significant shortest seedling length was observed in cultivar GF 2 (4.62 cm) followed by GF 11 (5.57 cm) and GF 12 (5.82 cm). The lowest inhibitory effect on seedling length was observed in cultivar AF 2 with the longest seedling length of 7.56 cm. A total of seven fungi significantly affected the seedling length during the assessment by the top of the paper method. Among them, *A. alternata* had shown the least effect on the seedling length and recorded longest seedling length (6.37 cm) among all tested fungi as compared to control. The shortest seedling length was recorded in seeds inoculated with *M. phaseolina* (3.95 cm) which was followed by *A. fumigatus* (5.45 cm) and *C. lunata* (5.64 cm). The present results are more or less similar to Suthar *et al.* (2014) [12]. They observed a reduction in seedling length of cumin when inoculated with culture filtrate of *Fusarium equiseti*. Further, they found the lowest root and shoot length at 50 per cent concentration (0.30, 0.28 cm) as compared to control (4.48, 2.66) in seeds of the GC 4 variety.

Effect of seed mycoflora on seedling vigour index-I

Seedling Vigour Index-I (SVI-I) influenced by individual seed mycoflora on the different cultivars of fennel are presented in Table 3. SVI-I which was worked out by multiplying the germination per cent with seedling length had revealed significant differences. Over the control, each of the seed mycoflora considerably reduced SVI-I of all five fennel cultivars. Cultivar GF 2 had recorded the least SVI-I (244.18) which was followed by GF 11 (368.33) and GF 12 (427.41). The maximum SVI-I was recorded in cultivar AF 2 (599.25) followed by AF 1 (472.17). Among all the culture filtrates of seven mycoflora tested for their effect on Seedling Vigour Index-I, The most toxic effect was induced by *M. phaseolina*, which resulted in the lowest SVI-I (195.60), which was followed by *A. niger* (362.86) and *A. fumigatus* (384.80), while *F. oxysporum* had the least negative impact and so had the highest SVI-I (469.12). The present findings are in harmony with the similar studies carried out by Lal *et al.* (2012) [9] and Suthar *et al.* (2014) [12]. Lal *et al.* (2012) [9] found that the seedling vigour index-I was reduced in seeds of ajwain treated with cultural filtrate of *Aspergillus flavus, A. niger, A. ochraceous, Drechslera australiensis, Fusarium sporotrichioides* and *Rhizoctonia oryzae.* Suthar *et al.* (2014) [12] also observed a reduction in seedling vigour index-I of cumin when inoculated with culture filtrate of *Fusarium equiseti*. They found the lowest SVI-I at 50 per cent concentration (8.70) as compared to control (656.88) in seeds of Gujarat Cumin 4 variety.

Table 1: Seed germination of fennel cultivars as influenced by seed mycoflora

Mycoflora	Seed germination (%)					Mean (F)
	GF 2	GF 11	GF 12	AF 1	AF 2	
Alternaria alternata	48.10	72.56	60.78	67.82	61.28	62.11
Macrophomina phaseolina	24.79	28.16	68.94	28.91	41.66	38.49
Fusarium oxysporum	56.06	68.34	65.04	51.58	82.53	64.71
Curvularia lunata	52.05	65.43	84.12	80.31	80.50	72.48
Aspergillus niger	52.54	72.79	48.54	60.13	67.29	60.26
Aspergillus flavus	32.13	41.63	84.88	75.88	73.38	61.58
Aspergillus fumigatus	52.03	76.75	60.14	76.21	84.10	69.84
Control	76.07	85.19	92.13	88.15	96.25	87.56
Mean (C)	49.22	63.86	70.57	66.12	73.37	64.63
	C		F		C × F	
S. Em.±	0.51		0.64		1.43	
C. D. at 5%	1.41		1.79		4.00	
C. V. %	4.42					

Note: C = Cultivar, F = Fungus

Table 2: Seedling length of fennel cultivars as influenced by seed mycoflora

Mycoflora	Seedling length (cm)					Mean (F)
	GF 2	GF 11	GF 12	AF 1	AF 2	
Alternaria alternata	3.73	6.83	6.10	7.31	7.90	6.37
Macrophomina phaseolina	2.97	2.53	3.80	2.69	7.75	3.95
Fusarium oxysporum	3.76	6.23	5.80	6.98	7.55	6.06
Curvularia lunata	3.55	5.42	5.90	7.08	6.25	5.64
Aspergillus niger	4.16	5.40	5.47	7.37	7.43	5.97
Aspergillus flavus	4.70	4.48	6.18	7.31	7.25	5.98
Aspergillus fumigatus	5.97	4.32	5.06	5.31	6.57	5.45
Control	8.08	9.36	8.23	9.01	9.75	8.89
Mean (C)	4.62	5.57	5.82	6.63	7.56	6.04
	C		F		C × F	
S. Em.±	0.05		0.06		0.14	
C. D. at 5%	0.13		0.18		0.40	
C. V. %	4.76					

Note: C = Cultivar, F = Fungus

Table 3: Seedling Vigour Index - I of fennel cultivars as influenced by seed mycoflora

Mycoflora	SVI-I					Mean (F)
	GF 2	GF 11	GF 12	AF 1	AF 2	
Alternaria alternata	179.53	501.12	364.43	467.96	620.25	426.66
Macrophomina phaseolina	78.43	64.62	425.45	76.79	332.70	195.60
Fusarium oxysporum	211.34	470.19	375.60	520.25	768.25	469.12
Curvularia lunata	185.12	345.15	555.28	586.05	500.25	434.37
Aspergillus niger	214.44	392.10	262.22	436.20	509.33	362.86
Aspergillus flavus	150.36	185.00	516.33	554.70	562.35	393.75
Aspergillus fumigatus	355.22	332.22	300.22	384.87	551.47	384.80
Control	579.00	656.25	619.75	750.55	949.36	710.98
Mean (C)	244.18	368.33	427.41	472.17	599.25	422.27
	C		F		C × F	
S. Em.±	3.99		5.04		11.28	
C. D. at 5%	11.16		14.12		31.58	
C.V.%	5.34					

Note: C = Cultivar, F = Fungus

Conclusion

All of the tested fungi had substantial inhibitory effects on seed germination of fennel cultivars and reduced seed germination. Among the five cultivars, GF 2 (49.22%) had the lowest seed germination percentage followed by GF 11 (63.86%) while the AF 2 cultivar had the highest seed germination percentage (73.37%). The maximum inhibitory effect on seed germination was of culture filtrate of *M. phaseolina* (38.49%). A total of seven fungi significantly affected the seedling length of all five cultivars. The shortest seedling length was observed in cultivar GF 2 (4.62 cm) followed by cultivar GF 11 (5.57 cm). The minimum seedling length was recorded in seeds inoculated with *M. phaseolina* (3.95 cm) while *A. alternata* had shown the least effect on the seedling length and recorded longest seedling length (6.37 cm). A similar inhibitory effect of all the seed mycoflora considerably reduced SVI-I of all five fennel cultivars. The maximum SVI-I was recorded in cultivar AF 2 (599.25) while cultivar GF 2 had recorded the least SVI-I (244.18). Culture filtrate of *F. oxysporum* had shown the least effect on the SVI-I (469.12) while *M. phaseolina* had the highest detrimental effect on SVI-I (195.60). Seed germinability, seedling length and SVI-I study revealed that among all fungi tested, *M. phaseolina* recorded the highest inhibitory effects.

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Conflict of interest: The authors declare that they have no conflict of interest.

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