Plant Archives Vol. 25, No. 1, 2025 pp. 2987-2991 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.no.1.429

IMPACT OF SEASONAL VARIATION ON MYCOFLORA DIVERSITY IN STORED CHIA (*SALVIA HISPANICA* L.) SEEDS: AN INVESTIGATION ON FUNGAL GROWTH PATTERNS

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(Date of Receiving- 14-01-2025; Date of Acceptance-15-03-2025)

ABSTRACT

The present study investigates the seasonal influence on mycoflora associated with stored chia (*Salvia hispanic*a L.) seeds. Chia seeds, known for their nutritional benefits are prone to fungal colonization during prolonged storage, which can lead to quality degradation and the potential production of mycotoxins. This study aimed to identify the mycoflora species present in chia seeds across different seasons. Samples of chia seeds were collected and stored over a year, with regular analysis to monitor fungal species diversity using culture-based methods. Results revealed significant seasonal variation in the composition and abundance of mycoflora, with higher fungal contamination observed during the rainy season while contamination was comparatively lesser during summer and winter. Fungal genera such as *Aspergillus*, *Penicillium*, and *Fusarium* were predominant, with variations in species dominance across seasons. Fungi with maximum recorded frequency was *Aspergillus flavus* in rainy (96.50%), in summer (93.75%) and in winter (92.50%) with high population of $24.50x10^2$, $21.0x10^2$ and $18.0x10^2$ in rainy, summer and winter seasons respectively. Whereas the minimum frequency was recorded for *Drechslera specifera* (5.39%) in rainy season, for *Epicoccum nigrum* it was 3.83% in summer season and for *Bipolaris bicolor* it was 4.17% in winter season.

Key words : Chia seeds, Mycoflora, Seasonal variation, Fungal contamination, *Salvia hispanica*, Mycotoxins.

Introduction

Chia (*Salvia hispanica* L.) seeds have gained significant global attention in recent years due to their exceptional nutritional properties (Rayes *et al*., 2008). Chia seeds are increasingly incorporated into human diets and food products, driving demand for their cultivation, processing, and storage (Ullah *et al*., 2016). However, like many stored seeds and grains, chia seeds are susceptible to microbial contamination, particularly from fungal species (mycoflora) that thrive under specific environmental conditions (Chauhan *et al*., 2015). Fungal growth in stored seeds is influenced by several factors, including temperature, humidity, moisture content, and storage duration (Magan and Aldred, 2007). These factors are subject to seasonal fluctuations, which can significantly affect the composition and proliferation of mycoflora on stored chia seeds (Saleh and Al-Delaimy, 2018). For instance, warmer and more humid conditions typically associated with summer months may promote the growth of heat and moisture tolerant fungal species (Nguyen *et al*., 2020), while cooler and drier conditions may suppress fungal activity or favour different species (Jayes and White, 2003). Understanding these seasonal dynamics is essential for developing effective strategies to prevent fungal contamination, ensuring both seed safety and quality during storage (Liu and Wu, 2010). Chia seeds also possess many medicinal properties which include function like blood pressure regulation (Ullah *et al*., 2016), glycemic control (Nieman *et al*., 2012), promotes regular bowel movement (Chicco *et al*., 2009), source of calcium and magnesium (Rayes *et al*., 2008), support bone density (Nieman *et al*.,2012), control type- 2 diabetes (Vuksan *et al*., 2010).

Fungi can degrade essential nutrients in chia seeds.

Mycotoxin produced by fungi causes increased cancer risk (Pitt and Hocking, 2009), unpleasant odour, changes in texture making seed less palatable (Magan and Aldred, 2007), decreased germination potential (Schimdt-Heydt *et al.*, 2009) thus reducing overall nutritional and economical value.

Materials and Methods

Sample collection

Chia seeds were sourced from local suppliers from Agra region and stored in a controlled environment. Samples were collected at different times points corresponding to seasonal changes Each seasonal sample consisted of 100 grams of chia seeds, stored in airtight containers to minimize contamination prior to analysis.

Isolation of mycoflora from samples

Fungal contamination was assessed using standard microbiological techniques. In this research a well-established seed plating method following the guidelines of ISTA (1966) was used, using Czapek's and Potato dextrose agar media (PDA).

Seed plating method

Petri dishes were sterilized to remove any microbial contamination by using autoclave and hot air oven, after those 200 seeds from each sample were taken and plated in sterilized petri dish at the rate of 20 seeds per plate. So, 20 plates from each sample are studied as two culture media are involved as mentioned above. These plates were then incubated for a duration of 7 days at 28±1°C. After incubation period these plates were examined and seed were studied under microscope and fungal colonies were identified and counted.

Fungal identification

Fungal isolates were identified based on their morphological characteristics. Recorded fungi were then sub-cultured and their identification was done with the help of Gilman (1957), Barnett (1960) and Subramanyam (1970). The frequency, abundance and total population of different fungal species were calculated by using following formula:

$$\text{Frequency} = \frac{\text{Total no. replicates in which fungal species was present}}{\text{Total no. replicates studied}} \times 100$$

$$\text{Abundance} = \frac{\text{Total no. of colonies of a particular fungus in all replicates}}{\text{Total no. of colonies of all the replicates}} \times 100$$

Total Population = Total no of colonies of a fungal species per gram sample.

Results and Discussion

Out of the total fungi recorded, *Aspergillus flavus* was present in maximum frequency in all three seasons (rainy 96.50%, summer 93.75%, winter 92.50%). In rainy season, minimum frequency was recorded for *Drechslera specifera* (5.39%). *Aspergillus montevidensis* was found to have least population ($1.40x10^2$). However, *Aspergillus chevalieri, Cercospora canescens, Cladosporium herbarum, Epicoccum nigrum, Paecilomyces fusisporus, Rhizoctonia solani* and *Trichothecium roseum* were not found associated with the seeds.

In winter season, lowest frequency of *Bipolaris bicolor* (4.17%) was reported with a population of 4.5×10^2. Minimum population was recorded for *Acremonium vitis* ($1.0x10^2$). However, *Acremonium glaucum, Aspergillus japonicus, A. montevidensis, A. nidulans, A. niveoglaucus, A. stellatus, A. sulphureus, A. tamari, A. sydowii, Botrytis cinerea, Cercospora canescens, Chaetomium spirale, Curvularia brachyspora, Epicoccum nigrum, Mucor circinalloides, Penicillium citrinum, Stachybotrys chartarum* and *Ulocladium chartarum* were not found associated.

In summer season, lowest frequency of *Epicoccum nigrum* (3.83%) was reported with a population of $2.0x10^2$. However, *Acremonium vitis, Aspergillus aculeatus, A. candidus, A. japonicus, A. montevidensis, A. niveoglaucus, A. quadrilineatus, A. terreus, A. sydowii, Bipolaris bicolor, Candida albicans, Cephalosporium aceremonium, Chaetomium spirale, Drechslera specifera, Fusarium oxysporum, Paecilomyces fusisporus, Penicillium digitatum, P. expansum, P. patulum* and *Ulocladium chartarum* were not found associated.

The results provide valuable insights into how various environmental conditions, specifically those associated with different seasons, affect the diversity and prevalence of fungal species and revealed significant seasonal variation in the composition and prevalence of mycoflora in stored chia seeds. Herrera-chan *et al*. in 2019 detected genus *Cladosporium, Phoma, Fusarium, Alternaria* and *Curvularia* on the superficial part of chia seeds. Presence of *Fusarium* spp., *Trichoderma* spp. and *Rhizopus* spp. were confirmed by Njeri *et al*. (2019). Jermnak *et al*. (2020) studied chia seeds in Bangkok and found 10 different mould species including *Aspergillus* and *Penicillium* species.

Table 1 : Quantitative determination of mycoflora in different environmental conditions associated with stored chia seed samples:

S. no.	Name of fungi	Winter			Summer			Rainy		
		%F	%A	P	%F	%A	P	%F	%A	P
1.	*Acremonium glaucum*	6	6	6	13.25	3.15	$12.65x10^2$	18.0	2.50	$6.25x10^2$
2.	*A. vitis*	7.20	1.55	$1.0x10^2$	6	6	6	8.10	1.80	$7.50x10^2$
3.	*Alternaria alternata*	46.98	1.72	$9.50x10^2$	50.00	5.30	$33.0x10^2$	46.0	2.50	$13.0x10^2$
4.	*Aspergillus aculeatus*	8.72	2.51	$3.0x10^2$	6	6	6	26.60	2.80	$3.50x10^2$
5.	*A. candidus*	7.46	2.38	$2.0x10^2$	6	6	6	17.50	2.25	$5.5X10^2$
6.	*A. chevalieri*	5.38	1.76	$1.50X10^2$	8.60	3.20	$2.20X10^2$	6	6	6
7.	*A. flavus*	92.50	3.00	$18.0X10^2$	93.75	3.20	$21.0X10^2$	96.50	3.10	$24.50X10^2$
8.	*A. fumigatus*	8.32	2.35	$11.0X10^2$	9.50	2.68	$3.40X10^2$	18.10	2.20	$11.50X10^2$
9	*A.. japonicus*	6	6	6	6	6	6	14.62	1.18	$1.80X10^2$
10.	*A. montevidensis*	6	6	6	6	6	6	8.28	1.20	$1.40X10^2$
11.	*A. nidulans*	6	6	6	62.90	2.60	$9.0x10^2$	22.50	2.45	$11.0x10^2$
12.	*A. niger*	68.48	2.42	$19.0x10^2$	71.25	2.18	$24.10x10^2$	78.20	3.00	$18.0x10^2$
13.	*A. niveoglaucus*	6	6	6	6	6	6	6.25	1.50	$4.10x10^2$
14.	*A. ochraceus*	24.30	3.00	$3.50x10^2$	51.38	3.25	$8.8x10^2$	62.25	3.15	$14.0x10^2$
15.	*A. paraciticus*	55.50	2.18	$11.32x10^2$	56.60	2.75	$31.35x10^2$	57.14	2.62	$13.78x10^2$
16.	*A. quadrilineatus*	4.38	1.43	$2.0x10^2$	6	6	6	7.46	1.84	$2.20x10^2$
17.	*A. stellatus*	6	6	6	6.60	1.80	$2.10x10^2$	7.80	2.15	$4.60x10^2$
18.	*A. sulphureus*	6	6	6	5.50	1.50	$2.40x10^2$	13.60	1.65	$2.80x10^2$
19.	*A. tamari*	6	6	6	14.20	1.60	$2.70x10^2$	16.50	1.80	$14.0x10^2$
20.	*A. terreus*	6.00	1.50	$3.0x10^2$	6	6	6	9.71	2.75	$8.80x10^2$
21.	*A. sydowii*	6	6	6	6	6	6	6.90	1.62	$6.0x10^2$
22.	*Bipolaris bicolor*	4.17	2.65	$4.5x10^2$	6	6	6	10.0	2.41	$7.08x10^2$
23.	*Botrytis cinerea*	6	6	6	4.38	1.80	$7.40x10^2$	9.38	2.42	$6.80x10^2$
24.	*Candida albicans*	5.35	1.60	$4.0x10^2$	6	6	6	8.90	2.34	$5.0x10^2$
25.	*Cephalosporium aceremonium*	6.86	2.51	$3.0x10^2$	6	6	6	8.14	1.85	$8.0x10^2$
26.	*Cercospora canescens*	6	6	6	9.09	2.68	$4.0x10^2$	6	6	6
27.	*Chaetomium globosum*	4.48	1.60	$1.20x10^2$	8.55	3.60	$14x10^2$	18.65	2.42	$7.0x10^2$
28.	*C. spirale*	6	6	6	6	6	6	11.25	2.68	6.0x102
29.	*Cladosporium cladosporoides*	14.10	1.80	$3.0x10^2$	15.50	2.10	$2.50x10^2$	20.10	1.35	$4.50x10^2$
30.	*C. herbarum*	22.25	1.48	$4.30x10^2$	4.0	2.20	$3.80x10^2$	6	6	6
31.	*Curvularia brachyspora*	6	6	6	4.62	1.14	$2.80x10^2$	7.20	3.80	$2.40x10^2$

Table 1 continued...

Table 1 continued...

32.	*C. lunata*	64.20	2.64	$13.0x10^2$	66.60	2.80	$14.50x10^2$	64.10	2.20	$8.30x10^2$
33.	*Drechslera specifera*	7.0	1.70	$11.0x10^2$	6	6	6	5.39	1.64	$5.50x10^2$
34.	*Epicoccum nigrum*	6	6	6	3.83	1.20	$2.0x10^2$	6	6	6
35.	*Fusarium moniliforme*	78.0	3.90	$14.50x10^2$	34.0	2.40	$14.0x10^2$	38.30	2.42	$9.0x10^2$
36.	*F. oxysporum*	26.24	2.30	$7.0x10^2$	6	6	6	14.40	1.80	$10.60x10^2$
37.	*F. solani*	16.32	1.25	$5.78x10^2$	12.24	1.98	$4.0x10^2$	24.21	3.20	$9.30x10^2$
38.	*Mucor circinalloides*	6	6	6	22.66	1.75	$13.20x10^2$	31.20	2.38	$7.0x10^2$
39.	*M. heamalis*	42.20	3.20	$18.0x10^2$	38.10	2.72	$12.0x10^2$	45.60	2.86	$15.0x10^2$
40.	*Paecilomyces fusisporus*	7.90	2.0	$6.50x10^2$	6	6	6	6	6	6
41.	*Penicillium chrysogenum*	45.60	2.70	$7.0x10^2$	52.15	2.42	$12.0x10^2$	55.20	3.80	$20x10^2$
42.	*P. citrinum*	6	6	6	51.35	2.85	$11.0x10^2$	46.12	2.38	$13x10^2$
43.	*P. digitatum*	6.24	2.37	$2.3x10^2$	6	6	6	11.48	1.41	$3.10x10^2$
44.	*P. expansum*	32.24	1.64	$8.0x10^2$	6	6	6	31.32	2.10	$7.20x10^2$
45.	*P. patulum*	7.46	1.91	$2.50x10^2$	6	6	6	11.61	2.83	$9.40x10^2$
46.	*Rhizoctonia solani*	6.32	1.80	$7.44x10^2$	7.80	2.10	$4.0x10^2$	6	6	6
47.	*Rhizopus stolonifer*	5.35	2.32	$8.10x10^2$	9.05	1.70	$10.0x10^2$	14.64	2.62	$5.0x10^2$
48.	*Stachybotrys chartarum*	6	6	6	8.46	2.48	$5.0x10^2$	10.09	2.65	$6.0x10^2$
49.	*Trichothecium roseum*	25.42	2.40	$16.70x10^2$	31.0	1.65	$24.10x10^2$	6	6	6
50	*Ulocladium chartarum*	6	6	6	6	6	6	15.62	1.75	$6.0x10^2$

F - denotes percentage frequency. A - denotes percentage abundance. P - denotes total population in terms of colonies per gram of samples.

Conclusion

Mycoflora diversity, particularly fungal species composition and density, was observed to fluctuate in response to changing environmental factors such as temperature and humidity across different seasons. During the warmer and more humid months, fungal growth was notably more abundant and diverse, which is consistent with previous studies that link higher moisture levels and elevated temperatures with increased fungal activity and aflatoxin production (Pitt and Hocking, 2009). This seasonal pattern can be attributed to the fact that most fungi thrive in environments with high humidity, which facilitates the germination and sporulation of fungal spores. Fungal genera such as *Aspergillus*, *Penicillium*, and *Fusarium* were the most dominant during these periods, which aligns with their known ability to proliferate in moist conditions.

The findings highlight the need for optimized storage strategies tailored to specific seasonal conditions to mitigate fungal contamination and preserve the quality of chia seeds. This research contributes to understanding the ecological dynamics of fungal communities in stored seeds and provides insights into improving post-harvest storage practices.

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