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Longevity of *Macrophomina phaseolina* in Stored Sesame Seeds and Impact on Biochemical Attributes of Diseased Seeds

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ABSTARCT

Introduction: Sesame, a versatile oilseed crop, holds a significant place in agriculture due to its nutritional value and economic importance. Its seeds are prized for their rich oil content and wide application in culinary and industrial sectors. *Macrophomina phaseolina* is a notorious seed-borne pathogen affecting sesame.

Objective: To find 1) Longevity of *Macrophomina phaseolina* in stored sesame seeds; 2) Biochemical changes in *M. phaseolina* infected sesame seeds.

Methods: The research investigated the one-year longevity of *M. phaseolina* in sesame seeds and revealed that infected seeds exhibited reduced germination and increased pathogen infection rates over time across diverse storage conditions.

Results: Storage at room temperature resulted in diminished germination (14-49%) and heightened infection (49-77%), whereas deep freeze storage displayed moderate germination (43-67%) and reduced infection (33-49%). The microsclerotia demonstrated viability for 12 months under both the conditions. Comparative biochemical analysis of infected and healthy seeds showcased lower moisture, overall oil content, and free fatty acids, along with elevated phenol content as a response to pathogen presence.

Conclusion: Seed borne nature *M. phaseolina* survived in sesame seeds up to 12 months and destroyed seed quality by decreasing seed moisture, oil and free fatty acid contents.

Keywords: Sesame; Seeds; Storage; *Macrophomina phaseolina*; Longevity; Biochemical properties; Seed borne

INTRODUCTION

Sesame (*Sesamum indicum* L.), an ancient and traditional oilseed crop originated in India and thrives in various climates across Asia, Africa, and South America. It is known as the "Queen of the Oilseeds" due to its high oil and protein content [1]. Its seeds are small, pearl-shaped and rich in nutrients, including antioxidants like sesamol. Its uses are multifaceted, including culinary, medicinal and ornamental applications across diverse cultures. Sesame oil with its stability and high smoke point is widely used in cooking, pharmaceuticals and cosmetics [2]. India is a major producer with about 18.1 lakh hectares dedicated to sesame yielding 8.1 lakh tons annually with Madhya Pradesh, Uttar Pradesh and Gujarat as key growing states [3].

Sesame cultivation faces significant challenges in terms of low yield and productivity with diseases being a major biotic factor responsible for these issues. Around 80 diseases caused by pathogens including fungi, bacteria, viruses and mycoplasma, afflict the crop leading to substantial quantitative and qualitative losses [4]. One of the most destructive among them is the root rot also known as seed rot or seedling blight disease caused by *Macrophomina phaseolina*. This disease prevails globally affecting a wide range of crops and can lead to yield losses ranging from 5 to 100 per cent [5]. Pathogen survives as sclerotia in soil and plant residues with a broad host range including staple food and oil crops. It induces wilting, stem and root blackening and pod rot.

The duration of viability for specific seed-borne pathogens relies on both the type of seeds they occupy and the pathogen's ability to maintain its viability and virulence when associated with seeds, spanning from one growing season to the following [6]. Studies on seed infection and pathogen survival highlight their detrimental impact on germination during long-term storage. *Macrophomina phaseolina* can remain infective for up to 20 months in seeds [7]. Sunflower seeds stored for 3.5 years failed to germinate due to *M. phaseolina* infection, leading to drastic changes in biochemical properties of heavily infected seeds [8]. Investigating over a year (November 2021 to November 2022), this study focused on *M. phaseolina* survival in sesame seeds (cv. Gujarat Til 3). Seeds were subjected to varying temperature conditions for specific durations as part of the experiment. Its effect on seed infection, seed germination and some biochemical constituents *viz*., moisture content, total phenol content total oil content and total free fatty acids were recorded at each two months interval.

MATERIALS AND METHODS

Sesame seeds (cv. GT 3) were procured from Agricultural research station, Amreli, Gujarat. Primarily seeds were observed under stereo binocular microscope for visual examination. Sesame seeds showing minute brown to black microsclerotia on the seed surface were selected for further storage. Naturally infected sesame seeds were preserved in plastic bags for 12 months to check the longevity of *M. phaseolina*. Seeds were subjected to two storage conditions i.e., Storage at room temperature and Storage in deep freeze (-20°C). To evaluate the impact of *M. phaseolina* on germination and infection, the upper paper method was employed. Germination paper was placed in a Petri plate and wetted with sterilized distilled water. Twenty-five seeds from each respective treatment were placed on the germination paper. The Petri plates were incubated in a seed germinator at 25°C for 7 days. At the end of incubation, number of seeds having *M. phaseolina* infection, germinated and rotten seeds were counted. Fresh healthy seeds without infection were considered as control. Per cent seed germination and per cent seed infection was calculated by given formula.

$$Percent\ seed\ ger\min ation = \frac{No.\ of\ \ germinated\ \ seeds}{Total\ \ no.\ of\ \ seeds\ \ observed} \times 100$$

$$Percent\ \ seed\ \ infection = \frac{No.\ of\ \ infected\ \ seeds}{Total\ \ no.\ of\ \ seeds\ \ observed} \times 100$$

The standard methodology for biochemical examination of infected stored seeds and fresh healthy seeds were followed for parameters *viz*., moisture, phenol, total oil and free fatty acid content.

Moisture content (%)

Change in seed moisture was determined by oven drying and is expressed in percentage dry weight [9]. Weight of empty Petri plate was taken. Then 5 gram of seeds in triplicate were weighed and kept in pre-heated oven at 100°C for 5 hours. After the cooling down of temperature, weight of dried seeds was taken. Per cent moisture was calculated by given formula.

$$Moisture\ content\ \ (\%) = \frac{W_1 - \ W_2}{W} \times 100$$

Here,

W1=Weight of dish + sample before drying

W2=Weight of dish + sample after drying

W =Weight of the sample

Total phenol content (mg/g)

A gram of seed sample was homogenized using a mortar and pestle in 80 percent methanol, with a final volume adjusted to 10 ml [10]. The mixture was then centrifuged for 10 minutes at 10,000 rpm and the resulting supernatant was collected. This extract was utilized for determining the total phenol content. The diluted extracts were placed in 10 ml glass test tubes and the total volume was brought to 1 ml using distilled water. Subsequently, 1 ml of Folin-Ciocalteau reagent (diluted 1:2 with water) was added followed by 1 ml of 20 percent Na_2CO_3. After 3 minutes tubes were incubated in a boiling water bath for 1 minute, then cooled and the total volume was adjusted to 5 ml using distilled water. The absorbance was measured at 650 nm. The phenol content was calculated utilizing a standard curve and expressed as mg/g of fresh weight.

$$\text{Phenol content (mg/g)} = \text{Graph factor} \times \text{O. D.} \times \frac{\text{Sample reading}}{\text{Weight of sample}} \times \frac{\text{Total volume}}{\text{Taken volume}} \times 10^{-4}$$

Total oil content (%)

Five grams of sesame seeds were ground and placed in a filter paper packet within a thimble [11]. The thimble was set up in a Soxhlet apparatus, and petroleum ether was used for a 4-hour extraction with gentle heating. After cooling, excess ether was evaporated and the remaining extracted oil was weighed using the given formula.

$$\text{Oil content (\%)} = \frac{\text{Weight of oil (gm)}}{\text{Weight of sample taken}} \times 100$$

Free fatty acids content (%)

One and half gram of extracted oil was taken in 150 ml conical flask and then 50 ml of methanol was added [12]. One ml of phenolphthalein was added and mixture was titrated with 0.1 N KOH until faint pink color persists. Titrate value was noted and amount of free fatty acid was calculated as per the formula given below.

$$\text{FFA content (\%)} = \frac{\text{Titre value} \times \text{Normality of KOH} \times 56.1 \times 1.98}{\text{Weight of sample (g)}}$$

RESULTS AND DISCUSSION

Longevity of *M. phaseolina* in sesame seeds during storage

The pathogen's longevity in stored seeds measured in context to the percentage of seed germination and seed infection after storage for various time periods. The germination of seeds stored at room temperature was ranged from 14 to 93 per cent as per different time period. Simultaneously seeds stored in deep freeze (-20°C) showed 43 to 95 per cent seed germination. Whereas per cent seed infection was ranged between 0 to 77 per cent and 0 to 49 per cent in seeds stored at room temperature and seeds stored in deep freeze (-20°C), respectively. In both the storge conditions, per cent seed germination of the infected stored seeds was significantly lower as compared to healthy seeds and per cent seed infection was also higher in stored infected seeds. Data revealed that with the increasing storage time period seed germination decreased and seed infection increased. For seeds stored at room temperature, the maximum seed germination observed after 2 months of storage was 49 per cent (Figure 1). However, this germination rate gradually decreased over time. After 12 months of seed storage the seed germination decreased to as low as 14 per cent. Additionally, the infection caused by *M. phaseolina* increased significantly reaching 77 per cent after 12 months of storage. In contrast, seeds stored in deep freeze conditions exhibited better preservation of seed germination. The maximum seed germination recorded for infected seeds after 2 months of storage was 67 per cent, which decreased to 43 percent after 12 months. The percentage of seed infection was also comparatively lower, ranging from 33 percent to 49 percent over the 2 months to 12 months storage period (Table 1) (Figures 1 and 2).

Table 1. Longevity of *M. phaseolina* in stored sesame seeds (cv. GT 3).

Storage period (months)	Seeds stored at room temperature		Seeds stored in deep freeze (-20°C)	
	Seed germination (%)	Seed infection (%)	Seed germination (%)	Seed infection (%)
0 (Fresh seeds)	93.00	0.00	95.00	0.00
2 (Jan-2022)	49.00	49.00	67.00	33.00
4 (March-2022)	36.00	59.00	60.00	36.00
6 (May-2022)	28.00	72.00	59.00	37.00
8 (July-2022)	25.00	67.00	54.00	45.00
10 (Sep-2022)	19.00	68.00	47.00	46.00
12 (Nov-2022)	14.00	77.00	43.00	49.00
S. Em. ±	1.23	1.69	1.89	1.06
C. D. at 5 %	3.63	4.97	5.55	3.14
C. V. (%)	6.55	6.04	6.23	6.08

Figure 1. Longevity of *M. phaseolina* in infected sesame seeds stored at room.

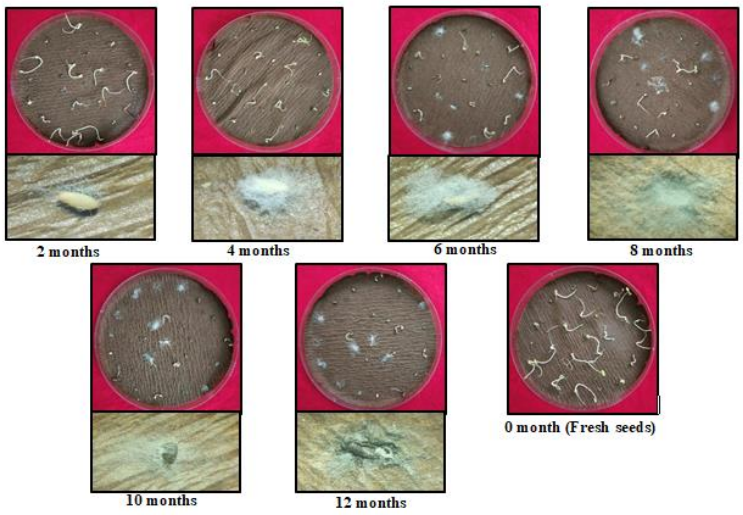
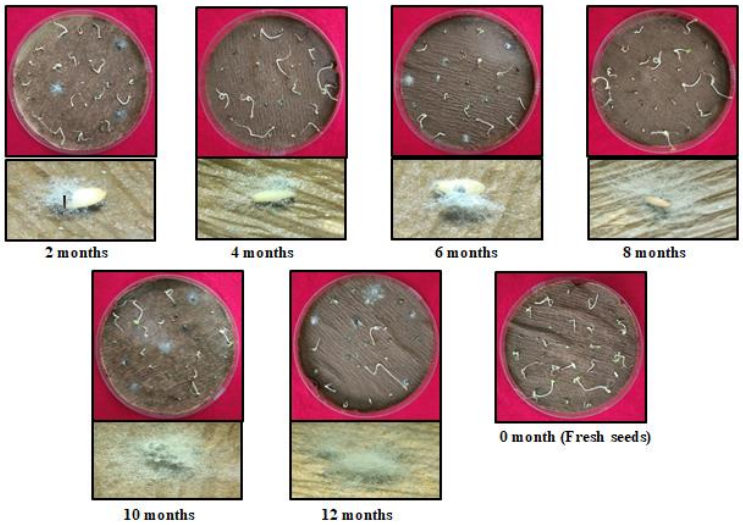


Figure 2. Longevity of *M. phaseolina* in infected sesame seeds stored in deep freeze (-20°C).



These findings indicate that infected seeds stored at room temperature resulted in a gradual decline in seed germination and an increase in pathogen infection over the time. On the other hand, deep freeze storage showed better preservation of seed germination and lower levels of pathogen infection compared to room temperature storage. The data presented in the study suggested that the longevity of *M. phaseolina*, increased over time during storage in both the conditions. It was observed that the microsclerotia of the fungus can remain viable for up to 12 months under storage conditions.

Correspondingly, observed the longevity and survival of *M. phaseolina* up to 12 months and effect on seed health in different storage temperatures (20°C, 4°C and -18°C) [13-15]. They found that the pathogen can survive even at -18°C over long term storage.

Biochemical examination

According to the findings presented in Table 2, the diseased seeds exhibited lower percentages of moisture, total oil content and free fatty acids compared to the healthy seeds. Conversely, the phenol content was higher in the

infected seeds. These results indicate that pathogen infection had a noticeable impact on the levels of moisture, oil and free fatty acids, while causing an increase in phenol content in the sick seeds. Moisture content was found optimum in healthy seeds (6.69 and 6.43%) during both the storage conditions. After twelve months of storage at room temperature, seeds got highly infected with lower moisture content (5.47 and 6.58%) as compared to healthy seeds. Seed moisture content was also found lowest (5.16 and 6.46%) during summer season and slightly increased during monsoon season. The healthy seeds exhibited a total phenol content of 7.11 mg/g in room temperature storage and 6.31 mg/g in deep freeze storage, surpassing the levels found in the infected seeds. During both storage conditions, an increase in the total phenol content was observed as the infection increases in infected seeds. The highest total phenol content of 10.51 mg/g was recorded in infected seeds stored for 12 months at room temperature, which coincided with 77 per cent infection rate of the pathogen. Healthy seeds exhibited a substantial quantity of total oil content, measuring 47.56 and 46.94 per cent more than the infected seeds in room temperature and deep freeze (-20°C) storage, respectively. In highly infected seeds total oil content was found low as compared to healthy seeds. In *M. phaseolina* infected seeds, total oil content was ranged from 33.30 to 43.56 per cent and 37.45 to 44.54 per cent in room temperature and deep freeze storage. Which was found reduced up to 10 per cent as compared to the healthy seeds in both the storage conditions. On the other hand, in seeds with a high infection rate, the total free fatty acids were found to be decreased drastically. After 12 months of storage total free fatty acids in sesame seeds was found 26.02 and 28.84 per cent in both the storage conditions. Whereas, healthy seeds contained a significantly higher free fatty acids, measuring 38.46 per cent in room temperature and 37.21 per cent in deep freeze (-20°C) (Table 2).

Table 2. Estimation of biochemical changes in sesame seeds in response to *M. phaseolina* (cv. GT 3) infection.

Seeds stored at room temperature					
Storage period (months)	Seed infection (%)	Moisture content (%)	Total phenol content (mg/g)	Total oil content (%)	Free fatty acids (%)
0 (Fresh seeds)	0	6.69	7.11	47.56	38.46
2 (Jan-2022)	49	7.13	8.27	43.56	35.77
4 (March-2022)	59	6.23	8.61	41.95	32.8
6 (May-2022)	72	5.16	10.34	39.11	31.12
8 (July-2022)	67	5.65	11.6	37.69	28.84
10 (Sep-2022)	68	6.13	11.02	34.65	27.71
12 (Nov-2022)	77	5.47	10.51	32.3	26.02
Seeds stored in deep freeze (-20 oC)					
Storage period (months)	Seed infection (%)	Moisture content (%)	Total phenol content (mg/g)	Total oil content (%)	Free fatty acids (%)
0 (Fresh seeds)	0	6.43	6.31	46.94	37.21
2 (Jan-2022)	33	6.85	7.52	44.54	36.2
4 (March-2022)	36	7.09	8.84	42.51	34.5
6 (May-2022)	37	6.46	9.98	41.87	33.37
8 (July-2022)	45	6.74	10.22	40.15	32.24
10 (Sep-2022)	46	6.7	10.16	39.78	29.41
12 (Nov-2022)	49	6.58	10.46	37.45	28.84

The results obtained in this study are in agreement with previous investigations carried out by, who also found significant reduction in moisture content, total oil and free fatty acids in *M. phaseolina* infected seeds [16-18]. Recorded gradual increase in total phenolic activity in contaminated sesame seeds [19].

CONCLUSION

Sesame (*Sesamum indicum* L.) is one of the major oilseed crops having the seeds as power house of energy. Seed health testing becomes crucial due to *M. phaseolina*'s seed-borne nature, as infected seeds can lead to heavy crop losses and impact human health when consumed directly. The longevity of *M. phaseolina* in sesame seeds by storing them for one year was carried out by seed germination and infection under different storage conditions. Seeds stored at room temperature (20-30°C) had lower germination rates, ranging from 14 to 49 per cent, compared to 93 per cent in healthy seeds, with infection levels between 49 to 77 per cent. In contrast, deep freeze (-20°C) storage showed germination rates of 43 to 67 per cent, compared to 95 per cent in healthy seeds, with infection levels between 33 to 49 per cent. The data indicated the significant impact of *M. phaseolina* on seed germination between infected and healthy seeds. The study also revealed the viability of microsclerotia for up to 12 months in both storage conditions. Further examinations on biochemical parameters of *M. phaseolina* infected stored sesame seeds in comparison to healthy seeds revealed that Infected seeds exhibited lower moisture (5.47 and 6.58%), total oil content (33.30 and 37.45%) and free fatty acids (26.02 and 28.84%) but higher phenol content (10.51 and 8.27 mg/g) compared to healthy seeds, which had (6.69 and 6.43%) moisture, (47.56 and 46.94%) total oil content, (38.46 and 37.21%) free fatty acids and (7.11 and 6.31 mg/g) total phenol content at room temperature and deep freeze storage, respectively

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