www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(12): 2682-2686

www.thepharmajournal.com
Received: 03-10-2023
Accepted: 12-11-2023

Dr. Ravina R Mevada
Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Dr. RG Parmar
Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Darshan M Parmar
Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Dr. DB Sisodiya
Department of Agricultural Entomology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Corresponding Author:
Dr. Ravina R Mevada
Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Longevity of *Macrophomina phaseolina* during various temperature storage conditions in sesame seeds and its impact on biochemical parameters of diseased seeds

Dr. Ravina R Mevada, Dr. RG Parmar, Darshan M Parmar and Dr. DB Sisodiya

Abstract
Sesame (*Sesamum indicum* L.) is a versatile oilseed plant that has an important position in agriculture due to its nutritional value and economic importance. Its seeds are valuable for their rich oil content and many uses in food and industry. *Macrophomina phaseolina* (Tassi) Goid. is one of the threatening pathogens affecting sesame seeds. The study looked on *M. phaseolina* one-year survival in sesame seeds. It was found that infected seeds exhibited reduced germination rates and greater pathogen infection rates over time under a range of storage circumstances, it was revealed. Room temperature storage resulted in lower germination (14-49%) and higher infection (49-77%), whereas deep freeze storage resulted in moderate germination (43-67%) and lower infection (33-49%). Under both conditions, the microsclerotia remained viable for 12 months. As a result of pathogen presence, comparative biochemical analysis of infected and healthy seeds revealed reduced moisture, total oil content, and free fatty acids, as well as higher phenol level. Seed-born nature *M. phaseolina* thrived in sesame seeds for up to 12 months, reducing seed moisture, oil and free fatty acid concentration.

Keywords: Sesame, seed borne, *Macrophomina phaseolina*, longevity, storage, biochemical properties

Introduction
Sesame (*Sesamum indicum* L.), an ancient and traditional oilseed crop from India, flourishes in a variety of climates throughout Asia, Africa and South America. It is referred to as the "Queen of the Oilseeds" because of its high oil and protein content (Bedigian, 2015) [4]. Its seeds are small, pearl-shaped, and high in minerals and antioxidants such as sesamol. It has a wide range of applications, including culinary, medicinal and ornamental purposes in various cultures. Sesame oil is frequently used in cooking, medicines and cosmetics due to its durability and high smoke point (Borchani *et al*., 2010) [6]. India is a major producer, with around 18.1 lakh hectares dedicated to sesame yielding 8.1 lakh tonnes yearly, with key growing states being Madhya Pradesh, Uttar Pradesh, and Gujarat (Anonymous, 2022) [2].
Sesame cultivation confronts substantial hurdles in terms of yield and productivity, with infections being a key biotic element to blame. Around 80 pathogen-caused illnesses, including fungi, bacteria, viruses, and mycoplasma, harm the crop, resulting in significant quantitative and qualitative losses (Vyas, 1981) [19]. One of the most damaging is root rot, also known as seed rot or seedling blight, which is caused by *Macrophomina phaseolina*. This disease is prevalent throughout the world, affecting a wide variety of crops and causing output losses ranging from 5% to 100% (Maiti *et al*., 1988) [11]. Pathogen lives as sclerotia in soil and plant wastes and has a diverse host range that includes staple food and oil crops. It causes wilting, blackening of the stems and roots, and pod rot.
The viability of various seed-borne diseases is determined by both the type of seeds they inhabit and the pathogen's capacity to retain viability and virulence when associated with seeds from one growing season to the next (Agarwal & Sinclair, 1997) [1]. Studies on pathogen survival and seed infection show that they have a negative impact on germination during long-term storage. *Macrophomina phaseolina* can survive in seeds for up to 20 months (Jitendra & Kumud, 2002) [9]. Because of *M. phaseolina* infection, sunflower seeds stored for 3.5 years failed to germinate. (Raut & Bhombe, 1984) [13], leading to drastic changes in biochemical properties of heavily infected seeds. This year-long study (November 2021 to November 2022) focused on *M. phaseolina* survival in sesame seeds (cv. Gujarat Til 3). As part of the experiment, seeds were treated to varied temperature conditions for varying lengths of time.

At each two-month interval, the effect on seed infection, seed germination, and some biochemical elements such as moisture content, total phenol content, total oil content, and total free fatty acids was observed.

Materials and Methods

1) *Macrophomina phaseolina* longevity in stored seeds of sesame

Sesame seeds (cv. GT 3) were obtained from the Agricultural Research Station in Amreli, Gujarat. Seeds were primarily examined visually using a stereo binocular microscope. For continued storage, sesame seeds with minute brown to black microsclerotia on the seed surface were chosen. To test the lifetime of *M. phaseolina*, naturally infected sesame seeds were stored in plastic bags for 12 months. Seeds were tested to two storage conditions: room temperature storage and deep freeze storage (-20 °C). The top paper method was used to assess the effect of *M. phaseolina* on germination and infection. In a Petri dish, germination paper was moistened with sterilized distilled water. On the germination paper, twenty-five seeds from each treatment were inserted. For seven days, the Petri plates were incubated in a seed germinator at 25 °C. At the end of the incubation period, the number of seeds infected with *M. phaseolina*, germinated seeds and rotting seeds were counted. Fresh, healthy seeds that were free of infection were used as controls. The given formula computed the percentages of seed germination and seed infection.

$$\text{Per cent seed germination} = \frac{\text{No. of germinated seeds}}{\text{Total no. of seeds observed}} \times 100$$

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

For standards such as moisture, phenol, total oil and free fatty acid content, the usual methodology for biochemical evaluation of infected stored seeds and fresh healthy seeds was used.

2) Biochemical changes in *M. phaseolina* infected sesame seeds Moisture content (%) (AOAC, 1995) [3]

Oven drying was used to determine the change in seed moisture, which is given as a percentage of dry weight. The weight of an empty Petri plate was measured. The seeds were then weighed in triplicate and placed in a preheated oven at 100 °C for 5 hours. The weight of dried seeds was measured once the temperature had cooled. The given formula was used to compute the percentage of moisture.

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

Here,
W_1 = Weight of dish + sample before drying
W_2 = Weight of dish + sample after drying
W = Weight of the sample

Total phenol content (mg/g) (Sadasivam & Manickam, 1992) [14].

One gram of seed sample was homogenized in 80 percent methanol with a mortar and pestle, and the final volume was adjusted to 10 ml. After centrifuging the mixture for 10 minutes at 10,000 rpm, the supernatant was recovered. This extract was used to calculate the total phenol content. Distilled water was used to dilute the extracts, which were deposited in 10 ml glass test tubes. Following that, 1 ml of Folin-Ciocalteau reagent (diluted 1:2 with water) and 1 ml of 20% Na2CO3 were added. After 3 minutes, the tubes were placed in a boiling water bath for 1 minute before being cooled and the total volume was corrected to 5 ml with distilled water. At 650 nm, the absorbance was measured. The phenol content was estimated using a standard curve and represented in milligrams per gram 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 (%) (Soxhlet, 1879) [17]

Five grams of sesame seeds were crushed and placed in a thimble-sized filter paper packet. The thimble was placed in a Soxhlet device, and a 4-hour extraction with mild heating was performed. Excess ether was removed after cooling, and the remaining extracted oil was weighed using the following formula.

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

Free fatty acids content (%) (Cox & Pearson, 1962) [7]

One and a half grams of extracted oil were placed in a 150 ml conical flask, followed by 50 mL of methanol. After adding one ml of phenolphthalein, the liquid was titrated with 0.1 N KOH until a faint pink hue remained. The titrate value was recorded, and the amount of free fatty acid was computed using the formula below.

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

Results and Discussion

1) *Macrophomina phaseolina* longevity in stored seeds of sesame

The pathogen's longevity in stored seeds was assessed in relation to the percentage of seed germination and seed infection after different time periods of storage (Table 1). Germination rates of seeds maintained at room temperature ranged from 13 to 92 percent depending on the time period. Simultaneous seed germination was observed in seeds maintained in deep freeze (-20 °C). Whereas seed infection ranged from 0 to 76% in seeds stored at ambient temperature and 0 to 48% in seeds placed in deep freeze (-20 °C), respectively. In both storage settings, the percentage of seed germination of infected stored seeds was much lower than that of healthy seeds, and the percentage of seed infection was similarly higher in stored infected seeds. Data showed that as storage time rose, seed germination reduced and seed infection increased. After two months of storage at room temperature, the highest seed germination observed was 48%. This germination rate, however, significantly reduced over time. Seed germination plummeted to as low as 13% after 12

months of storage. Furthermore, *M. phaseolina* infection rose dramatically after 12 months of storage, reaching 76%. Seeds maintained in deep freeze temperatures, on the other hand, preserved seed germination better. After two months of storage, the maximum seed germination rate for infected seeds was 66%, which dropped to 42% after a year. The percentage of seed infection was also reduced, ranging from 32% to 48% across the 2 months to 12 months storage period. (Fig. 1 & Fig. 2).

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

Storage period (months)	Seed storage			
	At room temperature		In deep freeze at (-20 ºC)	
	Seed germination (%)	Seed Infection (%)	Seed germination (%)	Seed infection (%)
0 (Fresh seeds)	92.00	0.00	94.00	0.00
2 (Jan-2022)	48.00	48.00	66.00	32.00
4 (March-2022)	35.00	58.00	59.00	35.00
6 (May-2022)	27.00	71.00	58.00	36.00
8 (July-2022)	24.00	68.00	53.00	44.00
10 (Sep-2022)	18.00	67.00	46.00	45.00
12 (Nov-2022)	13.00	76.00	42.00	48.00
S. Em. ±	1.22	1.68	1.88	1.05
C. D. at 5%	3.62	4.96	5.54	3.13
C. V. (%)	6.54	6.03	6.22	6.07

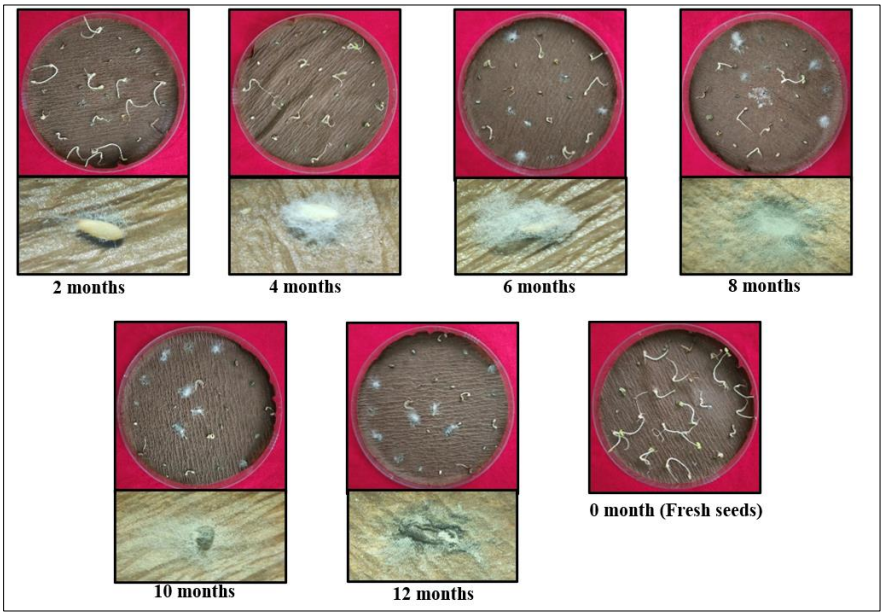

Fig 1: *Macrophomina phaseolina* longevity in infected sesame seeds stored at room temperature

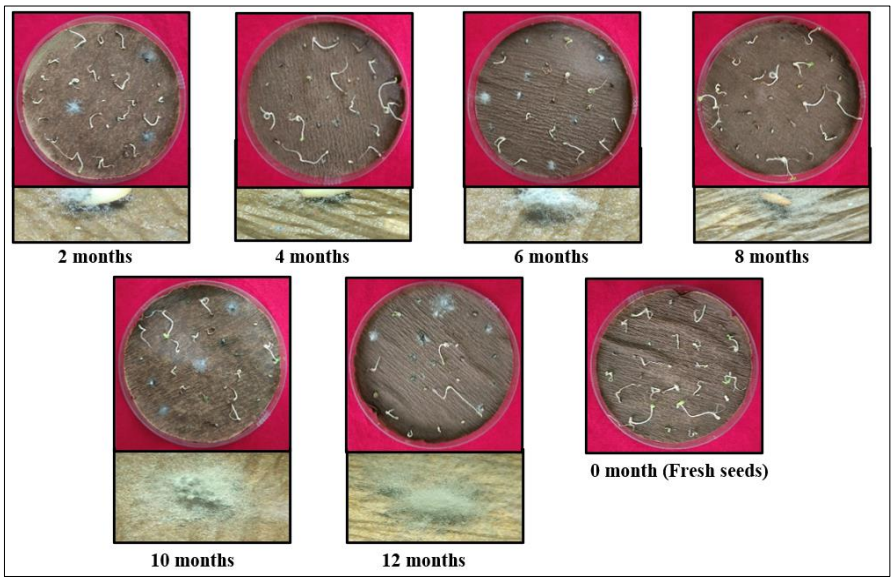

Fig 2: *M. phaseolina* longevity in infected sesame seeds stored in deep freeze (-20 ºC)

This study's data suggests that *M. phaseolina* longevity increased over time during storage in both conditions. It was observed that the fungus's microsclerotia can remain viable for up to 12 months under storage conditions. 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.

In line with this, a number of other researchers have also noted that *M. phaseolina* can survive for up to 12 months and that varying storage temperatures (20 °C, 4 °C, and -18 °C) can have an impact on the health of seeds. They discovered that the pathogen can endure long-term storage at -18 °C. (Kumar & Singh, 1984; Singh *et al*., 2003; Sultana *et al*., 2010) [10, 16].

2) Biochemical changes in *M. phaseolina* infected sesame seeds

The results shown in table 2 indicate that as compared to the healthy seeds, the diseased seeds had reduced percentages of moisture, total oil content, and free fatty acids. In contrast, the infected seeds had a greater phenol concentration. These findings show that pathogen infection increased the amount of phenol in the sick seeds while having a discernible effect on the amounts of moisture, oil, and free fatty acids (Table 2). Moisture content was found optimum in healthy seeds (6.68 and 6.42%) during both the storage conditions. After twelve months of storage at room temperature, seeds got highly infected with lower moisture content (5.46 and 6.56%) as compared to healthy seeds. Seed moisture content was also found lowest (5.15 and 6.45%) during summer season and slightly increased during monsoon season. The healthy seeds exhibited a total phenol content of 7.10 mg/g in room temperature storage and 6.30 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 10.50 mg/g was recorded in infected seeds stored for 12 months at room temperature, which coincided with 76 percent infection rate of the pathogen.

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	6.68	7.10	47.55	38.45
2 (Jan-2022)	48.00	7.12	8.26	43.55	35.76
4 (March-2022)	58.00	6.22	8.60	41.94	32.79
6 (May-2022)	71.00	5.15	10.33	39.10	31.11
8 (July-2022)	66.00	5.64	11.59	37.68	28.83
10 (Sep-2022)	67.00	6.12	11.01	34.64	27.70
12 (Nov-2022)	76.00	5.46	10.50	32.30	26.01
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	6.42	6.30	46.93	37.20
2 (Jan-2022)	32.00	6.84	7.51	44.53	36.19
4 (March-2022)	35.00	7.08	8.83	42.50	34.49
6 (May-2022)	36.00	6.45	9.97	41.86	33.36
8 (July-2022)	44.00	6.73	10.21	40.14	32.23
10 (Sep-2022)	45.00	6.69	10.15	39.77	29.40
12 (Nov-2022)	48.00	6.56	10.45	37.44	28.83

Healthy seeds exhibited a substantial quantity of total oil content, measuring 47.55 and 46.93 percent 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 32.30 to 43.55 percent and 37.44 to 44.53 percent in room temperature and deep freeze storage. Which was found reduced up to 10 percent 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 26.01 and 28.83 percent in both the storage conditions. Whereas, healthy seeds contained a significantly higher free fatty acids, measuring 38.45 percent in room temperature and 37.20 percent in deep freeze (-20 °C).

The results obtained in this study are in agreement with previous investigations carried out by different researchers, who also found significant reduction in moisture content, total oil and free fatty acids in *M. phaseolina* infected seeds (Mondal *et al*., 1981; Bhattacharya & Raha, 2002; Sagir *et al*., 1981). The gradual increase in total phenolic activity in contaminated sesame seeds was recorded earlier (Hassan *et al*., 2019) [12, 5, 15].

Conclusions

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* 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 13 to 48 percent, compared to 92 percent in healthy seeds, with infection levels between 49 to 77 percent. In contrast, deep freeze (-20 °C) storage showed germination rates of 42 to 66 percent, compared to 94 percent in healthy seeds, with infection levels between 32 to 48 percent. 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 (5.46 and 6.56%), total oil content (32.30 and 37.44%) and free fatty acids (26.01 and 28.83%) but higher phenol content (10.50 and 8.26 mg/g) compared to healthy seeds, which had (6.68 and 6.42%) moisture, (47.55 and 46.93%) total oil content, (38.45 and 37.20%) free fatty acids and (7.10 and 6.30 mg/g) total phenol content at room temperature and deep freeze storage, respectively.

Acknowledgement
This manuscript is part of doctoral research work. Hence, the authors are grateful to the Professor and Head, the Department of Plant Pathology, Anand Agricultural University, Anand, Gujarat for providing all the necessary facilities to conduct experiments.

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

References

1. Agarwal VK, Sinclair JB. Principles of seed pathology. 2nd ed. Boca Raton: CRC Press; c1997. p. 538.
2. Anonymous. Agricultural data. In: Agricultural Statistics databases [Internet]. 2022. [cited 2022 Jan 1]. Available from: http://faostat.fao.org
3. AOAC. Official methods of analysis. 16th ed. Arlington, VA, USA: Association of Official Analytical Chemists; c1995.
4. Bedigian D. Systematics and evolution in Sesamum: Evidence regarding the origin of sesame and its closest relatives. J Plant Taxon Geogr. 2015;70(1):1-42.
5. Bhattacharya K, Raha S. Deteriorative changes of maize, groundnut, and soybean seeds by fungi in storage. Mycopathologia. 2002;155:135-141.
6. Borchani C, Besbes S, Blecker CH, Attia H. Chemical characteristics and oxidative stability of sesame seed, sesame paste and olive oils. J Agric. Sci. Technol. 2010;12:585-596.
7. Cox HE, Pearson D. The chemical analysis of Foods. Chemical publishing company, New York; c1962. p. 420.
8. Hassan AB, Mohamed AI, Eikhatim KA, Elagib RA, Mahmoud NS, Mohamed MM, *et al*. Controlling fungal growth in sesame seeds with f-irradiation: impacts on some properties of sesame oil. Grasas Y Aceites. 2019;70(2):308.
9. Jitendra S, Kumud K. Location, survival, transmission and control of seed-borne Macrophomina phaseolina causing dry root rot and leaf blight in urdbean. Ann Plant Prot Sci, 2002, 10(1).
10. Kumar K, Singh J. Effect of fungicides on seed-borne fungi in sesame during storage. Seed Res. 1984;12:109-111.
11. Maiti S, Medge MR, Chattopadhyay SB. Handbook of annual oilseed crops. Oxford and IBII Publishing, Bombay, India; c1988. p. 131-133.
12. Mondal GC, Nandi D, Nandi B. Studies on deterioration of some oilseeds in storage I: variation in seed moisture, infection and germinability. Mycologia. 1981;73(1):157-166.
13. Raut JG, Bhombe BB. Longevity of Macrophomina phaseolina in sunflower seed. Indian Phytopathol. 1984;37:333-334.
14. Sadasivam S, Manickam A. Biochemical methods for agricultural sciences. Wiley Eastern Ltd., New Delhi; c1992. p. 187.
15. Sagir P, Sagir A, Sogut T. The effect of charcoal rot disease (Macrophomina phaseolina), irrigation and sowing date on oil and protein content of some sesame lines. J Turk Phytopathol. 2009;38(1):33-42.
16. Singh SD, Girish AG, Rao NK, Bramel PJ, Chandra S. Survival of Rhizoctonia bataticola in groundnut seed under different storage conditions. Seed Sci Technol. 2003;31:169-175.
17. Soxhlet F. The weight analytic determination of milk fat. Polytechnisches Journal. 1879;232:461-465.
18. Sultana N, Gul MS, Ghaffar A. Survival of fungi on seeds of bottle gourd, bitter gourd, and cucumber. Pak J Bot. 2010;42(3):1991-1997.
19. Vyas SC. Diseases in sesamum in India and their control. Pesticides. 1981;15:10.