Original Research Article

Effect ofpredominant seedmycoflora(*A. alternata*and *M. phaseolina*)on seed quality parameters of sesamum.

Abstract

Sesamum is an important oilseed crop affected by toxigenic fungal pathogens *viz.*, *Alternaria*, *Macrophomina*, *Fusariumand Aspergillus*spp. Among these, *A. alternata* and *M. phaseolina* are seed borne pathogens causing yield loss to an extent of 20 to 40 percent. The effect of *A. alternata*and *M*. *phaseolina* on seed quality parameters of susceptible cv. Swetha was investigated during 2022-2023. The *A. alternata* and *M. phaseolina* inoculated and uninoculated seeds of both test pathogens were tested for germination and seedling growth by rolled paper method and pot culture studies. Significant differences in seed germination, seedling length, seedling dryweight, seedling vigour index (SVI)I and II and seed moisture content were observed in susceptible cv. Swetha. The results indicated that *A. alternata*recorded ~~less~~ a lower seed germination percentage (60.50%), seedling length(656 cm), dry ~~weight~~mass (9.12 mg) and SVI-I and II (656 and 552respectively) over untreated seeds recording high germination (82.25%), seedling length(14.84 cm), dry ~~weight~~mass (13.17 mg) and SVI-I and II(1221 and 1116 respectively). Similar results were observed in *M.phaseolina* treated seeds where the pathogen treated seeds recorded ~~less~~ a lowerseed germination percentage (70.75 %), seedling length (9.42 cm), dry ~~weight~~mass(8.71 mg) and SVI-I and II (667 and 599respectively), than pathogen untreated seeds recording high seed germination (82.50 %), seedling length(14.25 cm), dry ~~weight~~mass (13.30 mg) and SVI-I and II (1175 and 1097 respectively).

Key words: *Alternariaalternata*, *Macrophominaphaseolina*, Sesamum, Germination, Seedling Vigour.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the important oilseed crops which is used as quality food, nutrition, edible oil, biomedicine and health care, all in one.The oil is used as the base for ayurvedic preparations and known as the Queen of oils. The crop is preferred due to its rich edible oil content (about 50%) and nutritious protein (about 23%) and having sufficient carbohydrates (15%) (Ranganatha *et al.*,2012). The crop is accepted~~on~~ worldwide due to its several medicinal benefits and it isalso rich source of linoleic acid, vitamin Eand vitamin B1 (Brar and Ahuja, 1979). The seeds contain~~s~~amino acids such asmethionine, tryptophan and ~~aminoacids with~~have innumerable health benefits. The therapeutic benefits of sesame seeds is

Comment [A1]: Should contain subheadings as per journal's guidelines: Different sub-sections, as given below, should be used

Comment [A2]: I know we all talk about weight when we need mass, but this IS a scientific report. From the internet: **The mass of a body is the amount of matter contained within it**, while the weight of a body is the force acting on it due to acceleration due to gravity.

Comment [A3]: Surely this is wrong. The seedling cannot be over half a meter long!

Comment [A4]: Subheadings should be numbered, as per journal guidelines: Starting from introduction with section 1. subsections should be numbered (for example 2.1 (then 2.1.1, 2.1.2, 2.2, etc.), up to three levels.

Comment [A5]: You will have to explain what this is. It will not be a common term for all your readers. I had to google it.

Comment [A6]: Reference

Comment [A7]: Wrong reference style. From the journal's author guidelines: References must be listed at the end of the manuscript and numbered in the order that they appear in the text. Every reference referred in the text must also present in the reference list and vice versa. In the text, citations should be indicated by the reference number in brackets [3].

Comment [A8]: What do you mean with 'accepted'? Rather 'consumed'?

mainly due to the presence of antioxidants like sesamin, sesaminol and sesamolin (Bedigian, 1985; Moazzami, 2006; El- Bramway and Mahesh, 2010).

Sesamum seeds are infected by different seed borne mycoflora. Infected seeds disperse mycoflora from seed to seed. They cause seed to deteriorate in the soil prior to germination, resulting in seedling mortality. Many fungi are known to be associated with seeds viz., *A. alernata*, *A. sesami*, *A. tenius*, *A. sesamicola*, *Macrophominaphaseolina*, *Aspergillus flavus*, *A. nige~~r~~*, and some *Fusarium* spp. Among these diseases, ~~at present~~ leaf spot/blight caused by *Alternaria~~alternata~~alternate and~~,~~*root rot caused by*Macrophominaphaseolina*arecurrently widespread and ~~posing~~ pose a~~m~~ajor threat~~s~~to the production and productivity of sesame crop throughoutIndia and with reference to Telangana state.They affect seed quality parameters, minimizing the seed germination and seedling vigour.

Materials and methods

The present investigation was carried out in Seed research and technology centre (SRTC), PJTSAU, Rajendranagar , Hyderabad.Sesamumseed samples collected from different districts of Telangana state were subjected to standard blotter method for the isolation of predominant seed mycoflora *A. alternata* and *M .phaseolina.*

Isolation of predominant seed mycoflora

For isolation of seed mycoflora associated with sesamum, seed samples were subjected to standard blotter method (ISTA, 2022)~~and~~ in orderto study theeffect of predominant seed mycoflora *A. alternata* and *M. phaseolina* on seed germination, seedling length, dry ~~weight~~ massand seedling vigour index. ~~the~~ The culture filtrate ~~is~~ was prepared as follows and data analysed (CRD).

a. Preparation of culture filtrates

The ten day old fungal growth of *M. alternata* and *M. phaseolina* on the separate agar plates was scrapedsampled by scraping. The spore suspension of the predominant fungi ~~were~~ was prepared by adding a sample of the colony scrapes to ~~using~~ sterile distilled water by mixing it with s vortex ~~mixture~~ mixerfor five minutes to separate spores from mycelia. The suspension was

Comment [A9]: Why repeat this?

Comment [A10]: often infected? Since they are surely not ALWAYS infected.

Comment [A11]: Since you have Alternaria and Aspergillus in the same sentence, I suggest writing out the genus name at least at the beginning of the list, otherwise it could lead to confusion.

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Comment [A12]: Capital letter if Nagar is part of the name. If not, you will have to explain. I for one do not know what it is, if not part of the name.

Comment [A13]: Sesamum is a genus of sesame, so capital letter and italics.

Comment [A14]: Not in reference list

Comment [A15]: What does CDR stand for? It is not a standard abbreviation.

filtered through autoclaved cheese cloth and the concentration of the spores ~~were~~ was adjusted to $1x10^9$ ml^{-1} by using a haemocytometer.

b. Seed inoculation

The healthy seeds of susceptible ~~*sesamum*~~ *Sesamum* cv. Swethatil were surface sterilized and soaked in spore suspension of both the pathogens viz., *A. alternata* and *M. phaseolina* for 30minutes and dried at room temperature ~~for over night~~overnight. The seed of control treatment were similarly treated except that they were soaked in sterile distilled water.

c. Rolled paper towel method

This method was used to determine the effect of seed borne inoculum on sesamum seed quality parameters, i.e., germination, seedling length, dry weight and seedling vigour. ~~According to ISTA 2022 rules hundred~~A hundred seeds of the previously *A. alternata* and *M. phaseolina* treated seed~~s~~ were selected randomly and placed in between two layers of moistened germination papers and carefully rolled. The rolled paper towels of both the pathogen treated seeds were kept in slanting positions and incubated for 7 days at 25± 2 °C. On the seventh day, the first count of germination was made. The seedlings that ~~are~~ looked morphologically normal were counted and germination was expressed as percentage.

d. **Germination percentage (%)**

On the seventh day, all normally developed seedlings were counted. Based on the number of normal seedlings, the germination percentage from each sample in each replication was computed.The following ~~formulae was~~formulae was used to compute the germination percentage (ISTA, 2022).

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

e. Seedling length (cm)

Comment [A16]: I presume?

Comment [A17]: Concentration singular

Comment [A18]: You need to say how

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Comment [A19]: Reference needed

Comment [A20]: Were these two fungi mixed in the treated seeds? Or did you have two sets of 100 seeds? Not sure from your description. If the treatments were separate, you will need to test a mix as well.

Comment [A21]: Germination papers or paper towels?And were these papers treated (autoclaved, microwaved) to ensure no inoculum from the air?

Comment [A22]: Since I believe you used a visual 'test'?

Ten normal seedlings from each replication were selected randomly on 7th day (~~Final~~ final count), for measuring shoot and root length.The shoot length was measured from the cotyledonary node to the tip of the apical bud. The root length was measured from the cotyledonary node to tip of the primary root. Themeanshoot and root length were expressed incentimetres (cm).

f. Dry ~~weight~~ mass of the seedling (mg)

The dry ~~weight~~ mass of the ten normal seedlings were selected for measurement of shoot length and root length were kept in butter paper bags and dried ~~in an hot air oven and maintained~~ at a constant temperature of 60°C. ~~temperature for 24 h. Later~~After 44 hours, the seedlings were removed and allowed to cool indesiccator for 30 min~~and then dry weight~~ after which the dry mass was recorded and expressed in milligrams (mg).

Comment [A23]: You started writing it with a space (see paragraph c) and should stick to one format.

g. **Seedling vigour index (SVI)**

Ten normally developed seedlings were ~~taken~~ randomly selectedfrom the germination test ~~at random and~~in order tomeasure~~the~~ root length and shoot length ~~was measured~~ in centimeters (cm). Vigour index was calculated by ~~the~~ the following formulae ~~as given by~~ (Abdul Baki and Anderson, ~~(~~1973).

Comment [A24]: Not in reference list

Vigour Index I = Seed germination (%) x ~~Seedling~~ seedling length (Shoot +Root length (cm))

Vigour index II= Seed germination (%)x ~~Seedling~~ seedling dry ~~wt~~ mass(mg)

Results

Table 1:Effect of *A. alternata* on seedand seedling quality in *~~sesamum~~ Sesamum*cv. Swetha~~by rolled paper towel method~~ during 2022 -2023

Comment [A25]: You are measuring seed (germination) and seedlings (length, SVI etc.) so you cannot call it seed quality.

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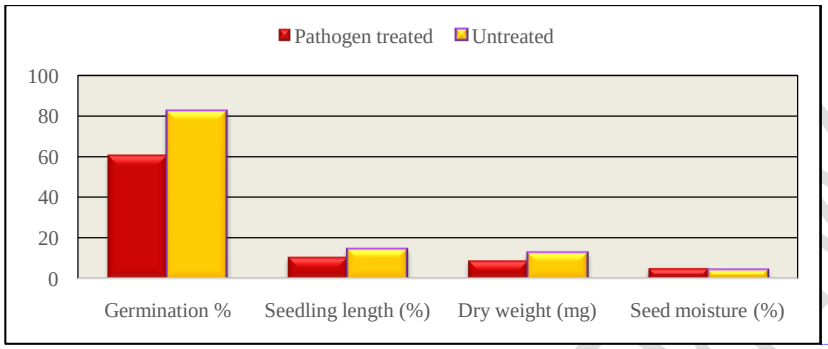
Comment [A26]: Remember – you are measuring seeds and seedlings, so your germination method is not important. You have mentioned the method in the materials and methods section.

S.No	*Alternaria alternata*	Germination (%)	Seedling length (cm)	Seedling vigour index (I)	Dry ~~wt~~mass (mg)	Seedling vigour index II	Moisture content (%)
1.	Pathogen treated	60.50 (51.04)	10.83	656	9.12	552	5.450 (13.49)
2.	Untreated	82.25 (65.07)	14.84	1221	13.17	1116	4.703 (12.50)
	Mean	**71.37**	**12.83**	**938.36**	**11.15**	**834**	**5.07**
	S.Em+	**0.95**	**0.27**	**26.93**	**1.19**	**23.95**	0.10

	CD at 5%	**3.29**	**0.95**	**93.19**	**0.33**	**84.52**	0.36



Comment [A27]: Change weight to mass. Seedling length – should it not be cm? You have % in figure

Figure 1:Effect of *A. alternata* on seed quality parameters in ~~*sesamum*~~ *Sesamum* cv. Swetha

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Plate 1. Germination of *A.alternata* treated and untreated seeds ~~in Rolled paper towel method~~

Comment [A28]: It is stilled called Figure. This should be Figure 2 and Plate 2 should be Figure 3.

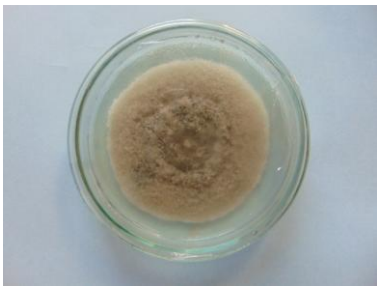

Plate 2. *Alternariaalternata*culture on PDA

Comment [A29]: Explain what it stands for. Not all your readers will be microbiologists/plant patholoigsts

Significant differencesin seed quality parameters were observed in susceptible ~~sesamum~~ *Sesamum*cv.Swetha which was artificially inoculated withconidial/mycelial suspensions of *A. alternata*~~along with~~compared to untreated seeds. The pathogen treated seeds showed significant differences in respect of germination percentage and treated seeds showed ~~less~~ a lower seed germination percentage (60.50%) over untreated seeds(82.25%). The mean germination percentage was 71.37%. Significant differences were observed between the seedling lengths of *A.alternata* treated and untreated seeds. The treated seeds recorded ~~less~~ lowerseedling length (10.83 cm) when compared to the untreated seeds (14.84 cm). The mean seedling length was recorded as 12.83cm.Incase of seed vigour index - I was 656 overuntreated seeds (1221). Pathogen treated seeds recorded mean dry ~~weight~~ mass of 9.125 mgand untreated seedsrecorded 13.175 mg. Seedling vigour index –II of untreated seeds was 1116 as compared with treated seeds(552). The pathogen treated seeds recorded higher seed moisture content (5.450%) than the untreated seeds (4.703%)~~.~~ (Table 1 and Fig 1).

The reduction in % seed germination might have been due to usage of energy rich compounds by the fungi that are otherwise required for proper germination and production of the enzymes and/`or toxins by the fungi (Irshad *et al*., 2017). This may be due to seed borne fungi like *Alternaria*, which is known to produce some toxins that are detrimental to seed germination (Rasmegowda and Naik, 2008). Similar reduction in germination, seed vigour, seedling blight and dry weight were reported by Bibi *et al*., (2023), Nayyar et al., (2014) in *Alternariaalternata* and Pravallika *et al*., (2023) in *Alternariasesami* when inoculated the ~~sesamum~~ *Sesamum*seeds with the pathogen.

Table 2: Effect of *M. phaseolina* on seed quality in ~~sesamum~~ *Sesamum*cv. Swetha~~by rolled paper towel method~~ during 2022 -2023

S. No	*M. phaseolina*	Germination (%)	Seedling length (cm)	Seedling vigour index (I)	Dry wt (mg)	Seedling vigour index (II)	Moisture content (%)
1.	Pathogen treated	70.75 (57.24)	9.42	667	8.71	599.30	5.410 (13.44)
2.	Untreated	82.50 (65.27)	14.25	1175	13.30	1097.50	4.720 (12.54)

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Comment [A30]: Along with seems as if you mixed the treated and the untreated seeds

Comment [A31]: In both treated and untreated seed? Though I fail to see why you need to know that. You want the difference between treated and untreated seed.

Comment [A32]: Treated or untreated seed? See above comment.

Comment [A33]: In treated seeds? And change "over' to "compared to".

Comment [A34]: This looks like a negative number which is impossible

Comment [A35]: The other percentages are only 2 decimals. Keep to one format. Doubt if 3 decimals have any real value.

Comment [A36]: Another negative??

Comment [A37]: Everything you report on here is past tense.

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	Mean	**76.62**	**11.83**	**921.09**	**11.00**	**848.40**	**5.06**
	S.Em+	**1.07**	**0.42**	**31.12**	**0.36**	**27.33**	0.13
	CD at 5%	**3.72**	**1.47**	**107.70**	**1.24**	**94.58**	0.45

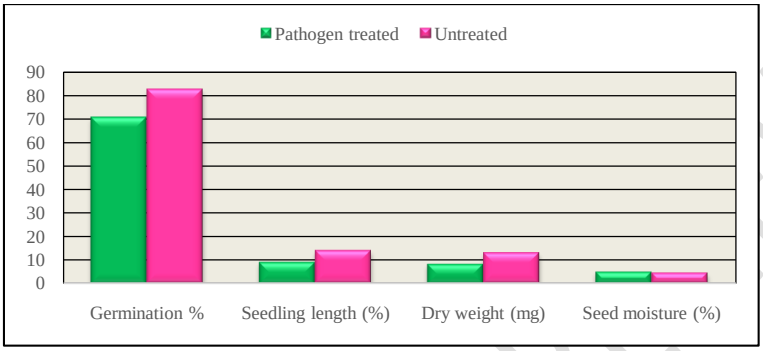

Fig 2 Effect of *M. phaseolina* on seed quality parameters in ~~*sesamum*~~ *Sesamum* cv. Swetha

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Plate 3. Germination of *M. phaseolina* treated and untreated seeds in ~~*sesamum*~~ *Sesamum* cv. Swetha~~by Rolled paper towel method~~ during 2022-23

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Plate 4.*Macrophomina phaseolina* culture on PDA

Significant differences were observed between treated and untreated seeds of ~~*sesamum*~~ *Sesamum*cv. Swetha. The treated seeds showed ~~less~~ a lowerseed germination percentage (70.75%) when compared to the untreated seeds(82.5%)~~with~~ while mean germination percentage was recorded as 76.62%.

The effect of *M. phaseolina*~~in~~ on the germination of artificially inoculated seeds of ~~*sesamum*~~ *Sesamum*cv. ~~swetha~~ Swethatilrecorded germination percentage(70.75%) as compared ~~with~~ tountreated seeds(82.5%). ~~In case of seedling~~ Mean seedling length of treated seeds was 9.42 cm~~and~~ whileuntreated seeds recorded14.25 cm. ~~Whereas seed~~ Seed vigour index I in treated seeds was 667 and the untreated seeds recorded SVI-I of 1175. Untreated seeds recorded mean dry ~~weight~~massof 8.710 mg~~and~~ while untreated recorded 13.30 mg. Seedling vigour index –II of treated and untreated seeds were observed. The untreated seeds showed ~~less~~ a higherSVI -II (1097.5) ~~as~~ when compared to treated seeds (599.3) (Table2 and Fig 2) The pathogen treated seed recorded ~~highest~~ higher seed moisture content (5.410%) than the pathogen untreated seeds (4.720%).

The reduction in seed germination might have been due to the enzymes and/or toxins produced by the fungi. Similar findings were reported by Lakhran *et al*., (2018) in chick pea and Rahman *et al*.,(2002) ~~Mungbean~~in mungbean.

Conclusion

~~Hence, from~~ From the results it is concluded that seed borne fungi *A. alternata* and *M. phaseolina* inhibit~~s~~ seed germination, seedling length, seedling dry ~~weight~~mass, and vigour of ~~sesamum~~ *Sesamum*seedlings ~~to a great extent~~significantly. ~~Hence farmers~~ Farmers are advised to take preventive measures including selection of sesame seeds from healthy fields followed by treating the seeds with recommended fungicides to achieve good germination, vigour, and optimum plant population in the field for realizing higher yields.

REFERENCES

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Comment [A39]: Why do you need this? You want to show the difference between treated and untreated.

Comment [A40]: Til??

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Comment [A41]: You never start a sentence with 'whereas".

Comment [A42]: Surely you mean higher? 1097 is more than 599.

Comment [A43]: A great extent is not scientific. Stick to significant or not significant.

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Comment [A44]: You never tested this, so how can you recommend it?

Comment [A45]: References should either be listed in chorological order (as per instructions for this journal) or alphabetically. Yours are neither.

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Comment [A46]: Which city and country?

Comment [A47]: Check the year. This is what I got on the internet: Brar, G. S. and K. L. Ahuja. "Sesame: its culture, genetics, breeding and biochemistry." (1980).

Comment [A48]: Please check this reference. The page numbers seem incorrect. Page 9-133? Impossible. I found this citation which could be the correct one.

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Comment [A50]: Not in text and you cannot cite an abstract only. You have to read the whole article and cite it as such.

Comment [A51]: 2014 in text

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