

Original Research Article

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Assessment of Combined Effect of *Macrophomina phaseolina* and *Fusarium oxysporum* on Disease Incidence of Sesame (*Sesamum indicum* L.)

B. Khamari^{1*}, S.K. Beura¹, A. Sushree¹ and S.P. Monalisa²

¹Department of plant pathology, College of Agriculture, OUAT, Bhubaneswar, Odisha, India

²Department of Seed Science and Technology, College of Agriculture,
OUAT, Bhubaneswar, Odisha, India

*Corresponding author

ABSTRACT

Stem and root rot and wilt diseases of sesame incited by *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *sesami* respectively were serious biotic constraints for sesame production. Investigations have been formulated on dual culture technique, cut stem inoculation experiment and soil inoculation experiment in order to assess the interaction and combined effect of *Macrophomina phaseolina* and *Fusarium oxysporum* f.sp. *sesami*. *Macrophomina* did not showed any antagonistic effect towards *Fusarium* and vice versa in dual culture experiment. Inoculation of healthy sesame stem with *Macrophomina*, *Fusarium* and in combination of *Macrophomina* + *Fusarium* revealed the colour of the stem changed from white to gray to black at different days of inoculations whereas the stem colour was green throughout the experimental period in control. The vascular bundle converted to dark and hollow stem in case of *Macrophomina* and *Macrophomina* + *Fusarium* when splitted 30 days after inoculation. Soil inoculation study revealed inoculation of *Macrophomina* + *Fusarium* recorded as low as 26.00% seed germination due to pre emergence damping off followed by in *Macrophomina* alone (seed germination 34%). The control pot recorded as high as 82% germination. *Macrophomina* is fast growing fungus as compared to *fusarium*, but the combination of *Macrophomina* and *Fusarium* didn't yield any antagonistic effect and found both in association leading to disease severity as compared to alone.

Keywords

Combining effect,
Fusarium oxysporum,
Interaction,
Macrophomina
phaseolina, Sesame.

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Introduction

Area and production of sesame is declining day by day in the traditional sesame growing areas due to severe biotic stresses such as Bacterial blight, *Macrophomina* stem rot, Phyllody, *Fusarium* wilt, Powdery mildew, *Alternaria* leaf spot and *Cercospora* leaf spot. *Macrophomina phaseolina* may cause heavy yield losses in sesame, if management is not proper. Variation of root rot disease incidence depends on the soil conditions and crop seasons. Wilt and root rot diseases of sesame

caused by *Fusarium oxysporum* f. sp. *sesami* and *Macrophomina phaseolina* are serious biotic constraints for sesame production. These are most important and widespread soil and seed-borne diseases of sesame grown where the climate is relatively dry and warm. Both *Macrophomina* and *Fusarium* are found associated with each other in sesame causing stem rot, root rot and wilt all together leading to rapid dying of the plant. Under the above circumstances an investigation has been

formulated to assess the synergistic or antagonistic relationship of *Macrophomina* and *Fusarium* under Bhubaneswar condition.

Materials and Methods

Macrophomina and *Fusarium* were isolated from the infected sesame plants and brought into pure culture following standard procedure of hyphal tip method.

Interaction study of *Macrophomina* and *Fusarium* in vitro

The interaction study between *Macrophomina* and *Fusarium* was done by dual culture technique keeping 5 mm mycelia disc of each pathogen at opposite side of the petriplates in triplicates. A set of individual test fungus was maintained as control. Observations were taken every day to see the interactions between them. A small tint of mycelia from interaction zone was taken in a slide and observed under microscope.

In vitro study of disease complex

In order to study the sole and combine effect on sesame stem, an *in vitro* experiment was undertaken. Two hundred ml potato dextrose broth was taken in 500ml conical flask. Two healthy sesame cut stem of 7.5 mm diameter with 10 cm length were kept in the flask and tightly plugged with non-absorbent cotton. Likewise 12 conical flasks were used for experiment. All the flasks were autoclaved.

After cooling, flask were inoculated with 6mm disc of *Macrophomina*, then *Fusarium* in second, both *Macrophomina* + *Fusarium* in third, fourth flask was remained uninoculated which was served as control. Three replications were maintained. These flask were then kept undisturbed and observations were taken regularly at 2, 4, 6, 10 and 30 days after inoculation. After 30 days, the stems

were taken out and splitted. The conditions of vascular bundle were recorded.

In vivo study of disease complex

The experiment was conducted in plastic pots. *Macrophomina phaseolina*, *Fusarium oxysporum* and both *Macrophomina phaseolina* + *Fusarium oxysporum* previously multiplied in sand maize medium were inoculated into sterilized soil at the rate of 2g/kg of soil. One of the treatment was retained uninoculated which served as control. All the pots were sown with seed variety VRI-1 and kept in completely randomized design. Each treatment replicated five times. Daily observations were undertaken and germination, disease incidence as well as mortality were recorded. The data obtained were analysed statistically.

Results and Discussion

Dual culture technique

Both the pathogen grew and intermingled with each other. *Fusarium* did not show any antagonistic effect against *Macrophomina* and vice versa. It was also observed that, *Macrophomina* grew faster than *Fusarium* covering maximum portion of the plate. Both the pathogens were coexisted while observed under microscope.

In vitro cut stem inoculation method

The sesame stem cuttings were inoculated with *Macrophomina*, *Fusarium* and in combination and the observations were recorded at 2, 4, 6, 10 and 30 days after inoculation. The colour of the mycelium was white and there was superficial coverage over the medium. The incidence was low in *Macrophomina* and *Fusarium* and the incidence was moderate in combination after 2 days of inoculation. At 4 days after

inoculation, the mycelium colour changes to grey, white and white + grey by *Macrophomina*, *Fusarium* and in *Macrophomina* + *Fusarium* respectively (Table 1).

The mycelium found to be grown upto half of the stem along with full coverage of the medium by *Macrophomina* and only coverage of the medium by the *Fusarium* without stem infection and both the combinations recorded full coverage of medium along with one fourth of the stem infection. Gradually the colour became dark grey covering total medium and the stem due to *Macrophomina* inoculation and creamy white in colour and covering total medium along with one fourth portion of the stem due to *Fusarium* and both combinations imparted grayish colour and covered full medium along with half portion of the stem after 6 days of inoculation. After 10 days of inoculation, the colour was found converted to black, creamy white and grey due to *Macrophomina*, *Fusarium* and combinations respectively covering the whole medium and stem by both the pathogen alone and in combination. After 30 days of

inoculation, the inoculated stem characteristics were studied before splitting and after splitting the stem into two halves. The inoculated stem before splitting the bark was dark in colour with some black dot like growths on *Macrophomina* and the stem was rotten with presence of whitish mycelia growth due to *Fusarium* and grayish colour in combination of *Macrophomina* and *Fusarium*.

After splitting, the stem colour was charcoal black with hollow vascular bundle, white with rotten vascular bundle and grey with hollow vascular bundle found inoculated with *Macrophomina*, *Fusarium* and combinations respectively. The microsclerotia formation was observed in *Macrophomina* and *Macrophomina* + *Fusarium* combinations. However, in control (without inoculation by pathogen) the stem colour was green throughout the experimental period.

It is revealed from the inoculation study that inoculation by *Macrophomina*, *Fusarium* and combination of *Macrophomina* + *Fusarium* the colour of the stem changed from white to gray to black at different days of inoculations.

Plate.1 Interaction between *Macrophomina phaseolina* and *Fusarium oxysporum* in dual culture techniques. **Plate.2** Cut stem inoculation experiment showing control, *Macrophomina*, *Fusarium*, *Macrophomina* + *Fusarium* (from left to right)

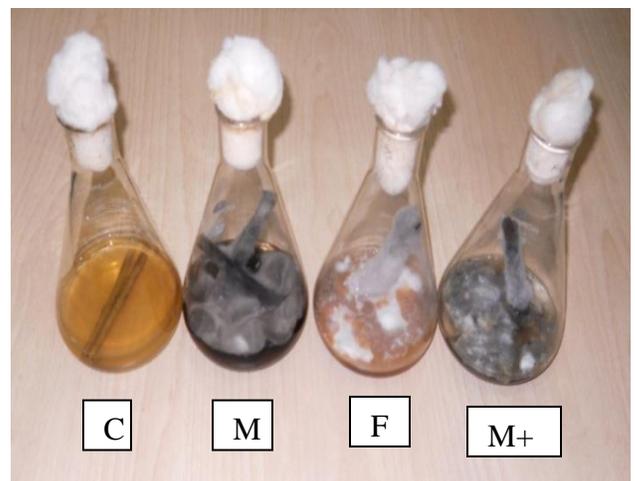
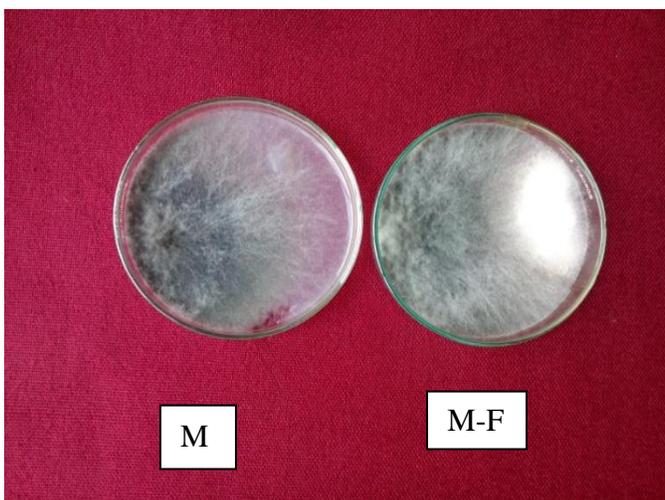


Table.1 Cultural characters of *Macrophomina*, *Fusarium* and their combinations at different days after inoculation

Treatments	2 DAI		4 DAI		6DAI		10 DAI	
	Colour	Coverage	Colour	Coverage	Colour	Coverage	Colour	Coverage
<i>Macrophomina</i>	White	Medium	Grey	Medium + half stem	Dark grey	Medium + stem	Black	Medium + stem
<i>Fusarium</i>	White	Medium	White	Medium	Creamy white	Medium + 1/4 th stem	Creamy white	Medium + stem
<i>Macrophomina</i> + <i>Fusarium</i>	White	Medium	White+ grey	Medium + 1/4 th stem	Grey	Medium + 1/2 stem	Grey	Medium + stem
Control	Green	-	Green	-	Green	-	Green	-

Treatments	30 days after Inoculation					
	Characteristics before splitting			Characteristics after splitting		
	Colour of medium	Colour of stem	Stem character	Colour of stem	Stem character	Microsclerotia
<i>Macrophomina</i>	Black	Black	Dark bark, fleshy, black growth on it	Black	Hollow VB	Present
<i>Fusarium</i>	Creamy white	Creamy white	Rotten, fleshy bark, whitish growth on it	White	Rotten VB	Absent
<i>Macrophomina</i> + <i>Fusarium</i>	Grey	Grey	Grey colour, fleshy	Grey	Hollow VB	Present
Control	Normal	Green	Green and normal	Light yellow	Normal VB	Absent

Table.2 Effect of soil inoculation of *Macrophomina*, *Fusarium* and their combinations

S. no	Treatments	Germination %	Pre emergence damping off
1	<i>Macrophomina</i>	34 (35.429)	66(54.534)
2	<i>Fusarium</i>	52 (46.311)	48(43.653)
3	<i>Macrophomina</i> + <i>Fusarium</i>	26 (27.586)	74(62.385)
4	Control	82 (65.332)	18(24.632)
SE(m)		5.020	5.022
CD		15.179	15.186

In vivo disease complex study

Under controlled condition, the soil inoculation study revealed inoculation of *Macrophomina* + *Fusarium* recorded as low as 26.00% seed germination due to pre emergence damping off corresponding to 72.00% followed by in *Macrophomina* alone

(seed germination 34%, pre emergence damping off 66%) and *Fusarium* recording 52% seed germination with 48% pre emergence damping off. However, the control pots recorded 82% germination without inoculation with any pathogen. Since, the activity of *Macrophomina* was found quick in respect of dual culture technique, cut stem

inoculation experiment and soil inoculation experiment as compared to *Fusarium*. It is proved that *Macrophomina* is fast growing fungus as compared to *Fusarium*. But the combination of *Macrophomina* and *Fusarium* didn't yield any antagonistic effect and found both in association leading to disease severity as compared to alone (Table 2).

The combined effect of *Macrophomina* and *Fusarium* was earlier studied by many workers in different crops. Mashooda *et al.*, (2005) observed that *M. phaseolina* and *Fusarium verticilloides* were responsible for collar rot, seedling rot and other diseases in okra.

They also observed that inoculated seed caused reduced seed germination as well as pre- and post-emergence mortality. *M. phaseolina* + *Fusarium oxysporum* results amaximum reduction of growth of brinjal and controlled the fungal complex with carbendazim significantly (Haseeb and

Archana, 2009). These findings were in agreement to the present investigation.

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