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Effect of inoculum load and duration of exposure to *Macrophomina phaseolina* on disease incidence of sesame

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Abstract
An experiment was conducted to critically evaluate the impact of inoculum load as well as duration of exposure to pathogen on disease incidence. The maximum germination percentage (27.5%), minimum seed rot (72.5%), maximum seedling mortality (20%) was found at lowest inoculum level of 2g/kg. In contrast to that, lowest germination% (12.5%), maximum seed rot (87.5%), less seedling mortality (12.5%) and no survival of plants was obtained at high inoculums level of 10g/kg of soil. Seed soaking of 24 hours in culture filtrate registered minimum germination (5.0%), maximum diseased seed (95%), short seedling length (3.8 cm) and low vigour index (18.6). The lowest hour of seed soaking of 4 hours recorded maximum germination (11.0%), minimum disease (86.0%), high seedling length (5.9cm) and high vigour index (64.0). It is evident from experiment that, Germination per cent of test plant was gradually decreased with increasing inoculum level and duration of exposure to pathogen. The seed rot and seedling mortality increases with increasing inoculums load. It also affects the survival of seedling as well as reduce seedling length and vigour index.

Keywords: Culture filtrate, *Macrophomina phaseolina*, Pathogenicity, inoculums density, stem and root rot

Introduction
Sesame (*Sesamum indicum* L.) is an important oilseed crop grown in hotter and drier areas. It is a quality food, nutrition, edible oil, bio-medicine and health care. Because of its high oil yield, mildness, high nutritive values and pleasant taste, it is known as "queen of oilseed crop". It is widely cultivated oilseed crop. India is the world leader with the maximum (25.8%) production from the largest (29.8%) area and highest (40%) export in the world. India produces 870 thousand metric tons in fiscal year 2015-2016 with productivity of 413 kg/ha (Annual report, 2016-17) [2]. Now days, area and production of sesame is declining due to lack of wider adapting cultivars, shattering of capsules at maturity, non-synchronous maturity, poor stand establishment, lack of fertilizer responses, profuse branching, and low harvest index (Ashri 1994). Besides these, extreme susceptibility to biotic and abiotic stresses also a major production limiting factor. The stem and root rot disease is a major disease of sesame occurs from seedling stage to maturity stage causing around 5–100% yield loss (Vyas, 1981). *Macrophomina phaseolina*, the incitant of the disease is polyphagous, cosmopolitan, seed borne as well as soil borne in nature. The pathogen survives in soil for longer duration. The rate of disease development and disease severity depends on the pathogen propagules present in the soils (Madden, 1980; Kenerley & Bruck, 1987) [8, 6]. Similarly soaking of seeds with pathogen culture filtrate also causes severity of disease. The severity of disease depends on inoculums load as well as the duration of exposure to the pathogen. Keeping in view, the present investigation was initiated to critically evaluate the impact of inoculum load as well as duration of exposure of seed to pathogen culture on disease incidence.

Materials and methods
Naturally infected plant samples were collected and the pathogen was isolated using standard technique and maintained in PDA slants. The pathogen was identified as *Macrophomina phaseolina* (ID No. 9811.15) by ITCC, IARI, New Delhi.

Mass culturing of pathogen inoculum

In order to prepare large quantity of *M. phaseolina* inoculum, sand+maize meal substrate was used by mixing 95 gm of sand and 5 gm of maize meal thoroughly and moistened with 10 ml sterilized water. The substrate was transferred into 250 ml conical flask and was sterilized in the autoclave at 15 psi for 20 minutes. It was left for 24 hours for cooling and then 5 mm disc of *M. phaseolina* from actively growing culture was transferred to the conical flask. It was incubated at room temperature for 4-6 weeks with daily shaking in order to achieve uniform multiplication of the pathogen inoculum. After 4-6 weeks, the color of the substrate in the conical flask turned black due to the sclerotial formation.

Effect of inoculum load on germination and disease development

To determine the effect of different amount of inoculum of *M. phaseolina* on plant growth and disease development, the experiment was conducted. For this purpose, already prepared inoculums of *M. phaseolina* in sand maize meal were added in 1kg of sterilized soil filled in Plastic pots @ 2g, 5g, 8g and 10g. Pots without inoculum was also maintained which served as control. It was kept for three days for proper multiplication and colonisation. Each pot was sown with ten surface sterilized sesame seeds equidistance to each other. The experiment was laid in completely randomized block design with five treatments and four replications. Plants were observed daily for seed germination as well as plant mortality.

Disease assessment

Disease incidence expressed as seed rot and seedling mortality was determined at 7 and 15 days after sowing respectively and per cent healthy survived seedlings were also determined. The following formulae were used for determining these disease criteria.

Seed rot % = No. of infected seeds / No. of seeds sown X 100
Seedling mortality % = No. of dead seedlings / No. of sown seeds X 100
Survived seedlings % = No. of seedlings survived / No. of sown seeds X 100

Effect of culture filtrate on seed germination

In order to study the effect of culture filtrates, healthy and surface sterilized sesame seeds of variety Amrit were choosen. Twenty days old culture of *Macrophomina phaseolina* grown in potato dextrose broth were filtered and filtrate was collected after passing through whatman no 1 filter paper. Surface sterilized seeds were subjected to different hours of soaking in culture filtrate such as 0, 4, 8, 12, 16, 20 and 24 hours and there after plated using standard blotter paper method (ISTA). On the 6th day (last count), number of healthy and diseased seeds were counted and percentage was calculated. Seedling length of five normal seedlings was measured and vigour index was calculated using formulae.

Seed vigour index- I = Mean seedling length (cm) x Germination (%)

Result and discussion

The impact of inoculum load and duration of exposure to pathogen on severity of the disease was studied with two methods. The result obtained from the experiments are depicted in table 1 and table 2.

Effect of different levels of inoculum load

The germination count was taken at 6th day of sowing and germination percentage was calculated. From the table1, it was seen that the germination percentage varied in between 12.5 to 27.5% in various levels of inocula while in control the corresponding figure was 80%. Among the treatments, maximum germination percentage of 27.5 was registered at inoculum level of 2g/kg, which was 65.62% reduction over control. The next best in the order of merit is 5g/kg registering 25.0% of germination followed by 8g/kg (17.5%). The lowest germination per cent was obtained at inoculums level of 10g/kg which was 84.37% reduction over control. All the treatments were at par and varied significantly from control which registered as high as 80%.

With respect to seed rot or pre emergence damping off, it was evident that minimum seed rot of 72.5% was recorded at lower level of inocula (2g/kg) followed by at 5g/kg of inocula (75%). Inoculum levels of 8g/kg and 10g/kg harboured 82.5% and 87.5% respectively. However, 20% of seed rot was registered in control. The seed rot per cent was found inversely related to the pathogen inoculums load.

Post emergence damping off was recorded at 15th day after sowing. Maximum infestation (20%) were registered in 2g/ kg and 5g/kg followed by 8g/kg (15%) and less in 10g/kg (12.5%). All the treatments were found at par with each other. The maximum plant mortality and root infection combinely was recorded in treatments where the pathogen inoculum was high i.e. 10g/kg followed by 8g inoculum/kg of soil. It shows that the disease severity depends on pathogen load.

The survival per cent was calculated basing on pathogen survived after 30days of sowing. The survival per cent was maximum in lowest inoculum levels i.e. 2g/kg (7.5%) followed by 5 g/kg (5.0%) which is 90% and 93.75% reduction over control. No plants survived at high dose of inoculum i.e. 10g/kg of soil. All the plants collapsed at 30th day of sowing. It is clearly evident from table 1 that impact of *M. phaseolina* on inoculated sesame plants was increased with increasing inoculum density. Germination per cent of test plant was gradually decreased with increasing inoculum level. The seed rot and seedling mortality increases with increasing inoculums load. It also affects the survival of seedling. Soil application of inocula resulted in seed rot and reduced seed germination.

Effect of different hours of seed soaking on germination

There was marked variation in germinability of sesame seed with different hours of seed soaking in culture filtrate of *M. phaseolina*. It was evident from table 2 that, 24 hours of seed soaking registered minimum germination (5.0%) corresponding to maximum diseased seed (95%). It also reduced the seedling length (3.8 cm) and hence the vigour index (18.6) followed by 20 hrs of seed soaking which was at par to it. The maximum germination per cent was recorded in control (81%) with minimum disease per cent (2.0), maximum seedling length (6.2 cm) and vigour index (504.2). The next best in the order of merit was 4 hours of seed soaking followed by 8 hours of seed soaking which were at par. From the experiment it was seen that the germination percentage was gradually decreased with increasing time of seed soaking. Similarly the diseased percentage was found increased and it also affects the seedling length. Hence, the vigour index was also affected. There might be presence of toxic metabolites in culture filtrate which caused inhibition in seed germination. Hence, reduced the vigour index.

Similar result was obtained by McCain & Scharpf (1989) [9], Dawar and Ghaffar (1998) [4] and Moradia (2011) [10] and Umamaheswari *et al*. (2001) [11]. They stated that increase in sclerotial population of *M. phaseolina* increased the infection and colonization in sunflower, groundnut and conifers respectively. Khanzada *et al* (2012) [7] reported soil infestation method comparatively checked more plant growth of okra than seed infestation method. Seed germination, plant growth, plant mortality and root infection of okra plants were adversely affected with the increasing inoculum levels of *M. phaseolina*. These conclusions of earlier workers draw enough support for present finding. Germination percentage was gradually decreased with increasing time of seed soaking. It also reduced the seedling length and the vigour index. The present finding is in line of conformity with earlier findings of Akhtar *et al.* (2011) [1] who found *M. phaseolina* was pathogenic against 18 test plant species and its seed infection efficiency was 100% with significant reduction in seedling vigour index.

The disease intensity and severity depends on inoculums of load and duration of exposure to the pathogen. So there is a need to reduce the pathogen inocula for a better and healthy crop.

Table 1: Effect of different levels of *M. phaseolina* inoculum on disease development

Treatments (g/kg)	Germination (%)	% reduction over control	Seed rot (%)	Seedling mortality (%)	Survival (%)	% reduction over control
2	27.5 (31.38)	65.62	72.5 (58.58)	20.0 (25.65)	7.5 (13.82)*	90.62
5	25.0 (29.72)	68.75	75.0 (60.24)	20.0 (26.55)	5.0 (6.64)	93.75
8	17.5 (21.20)	78.12	82.5 (68.76)	15.0 (16.59)	2.5 (4.61)	96.87
10	12.5 (17.88)	84.37	87.5 (72.08)	12.5 (17.88)	0.0 (0.00)	100
Control	80 (63.78)	-	20 (26.18)	0 (0.00)	80 (63.78)	-
SE(m)± CD(0.05)	5.094 15.493		5.098 15.508	5.570 16.944	4.374 13.304	

*****Data in the parenthesis indicates the transformed values

Table 2: Disease development by seed soaking in culture filtrate as influenced by different hours

Sl no	Time of seed soaking (hr)	Germination (%)	Diseased seed (%)	Seedling length (cm)	Vigour index
1	Control	81.0	2.0	6.2	504.2
2	4	11.0	86.0	5.9	64.0
3	8	10.0	85.0	5.6	53.9
4	12	8.0	88.0	5.0	41.5
5	16	8.0	90.5	4.8	38.9
6	20	6.0	94.0	4.0	23.6
7	24	5.0	95.0	3.8	18.6
SE(m)± CD(0.01)		1.874 5.547	2.246 6.650	0.344 1.017	21.118 62.526

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