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Survey and occurrence of stem and root rot of sesame in different districts of Rajasthan

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Abstract
Sesame (*Sesamum indicum* L.) is oldest oil seed crop and affected by many diseases, in which *Macrophomina phaseolina* pathogen causes heavy yield losses during cropping season. It caused stem and root rot and prevalent on all plant parts and pathogen is saprophytic and soil borne, so management is difficult in nature, therefore is a major problem of sesame due to its wider host range and survive in soil in the form of sclerotia. The disease incidence was (23.84 - 53.72%) recorded during survey in major sesame growing areas of Rajasthan. Disease incidence was found maximum in Jodhpur district (41.14%) followed by Pali (38.71%), Jaipur (35.90%), Nagaur (28.58%) and lowest incidence was observed in Tonk district (24.41%). Mean disease incidence was 33.74%. Pathogen was isolated from infected stem and root parts and purified by using hyphal tip cut method. Pathogenicity was proved through Koch's postulates by using seed cum soil inoculation techniques and 53.84% disease incidence.

Keywords: survey, occurrence, stem, root rot, sesame, *Sesamum indicum* L.

Introduction
Sesame (*Sesamum indicum* L.) is valuable and edible seed crop belongs to family *Padaliaceae.* In India, it is grown from ancient times (Weiss, 1971) [24]. It is generally called as "Til" and popularly known as "Queen of oilseeds". It has good quality parameters and resistance to oxidation (Bedigian and Harlen, 1986). Sesame seed enriched with 50 per cent edible oil, 20 per cent protein and contains about 39 per cent linolenic acid and 47 per cent oleic acid. (Shyu and Hwang, 2002) [20]. Sesame oil cake contains average 32 per cent crude.
Seed is highly rich in quality proteins and essential amino acids, especially methionine which is considered as rejuvenative and anti-aging for human body. Sesame oil is useful for soap making, skin care industries, health food industries and cosmatic purpose. Sesame oil is cholestrol free and stable doesn't form rancid. Its seed used as sweets making and medicinal forms. Sesame varieties are both white and black seeded. White seeded varieties used for bakery products and black seeded varieties used for medicinal purpose. In South India sesame oil used for cooking. Sesame ranked first among oil seed crops in oil content (50-52%) with significant dietary energy (6335 kcal per kg) (Kumar and Goel, 1994) [14]. Sesame mainly grown under tropical and subtropical regions of India with an annual acreage of around 19.17 lakh ha and production of about 8.66 lakh tonnes with 413 kg/ha productivity (Anonymous, 2017a) [2]. The dominant sesame growing states are Madhya Pradesh, Uttar Pradesh, Rajasthan, Odisha, Gujarat, Karnataka, Chhattisgarh and Maharashtra (Anonymous, 2017a) [2]. In Rajasthan, sesame occupied 2.51 lakh ha area with total production of 67864 MT and average productivity of 269 kg/ ha (Anonymous, 2017b) [3]. Main sesame growing districts of Rajasthan are Pali, Sawai Madhopur, Sirohi, Jodhpur, Bikaner, Karauli, Jalore, Bhilwara, Churu, Dausa, Tonk, Nagaur, Ajmer, Bundi and Kota. Decline in sesame area and productivity are due to attributed to poor crop management and severe biotic and abiotic stresses in Rajasthan.Many pests and diseases infect sesame crop from seedling stage to till harvesting time. Among all fungal, bacterial, viral and phytoplasmal diseases, stem and root rot incited by *Macrophomina phaseolina* is a major disease and causes severe losses in seed yield. Initial symptoms of stem and root rot was yellowing of leaves and plants wilt within a week. Diseased plant does not pull out easily. The tissue at collar region ruptures and becomes soft. After infection, dark brown coloured lesion seen on the stem at ground level. Black coloured dot like structure (sclerotia) may be seen on stem, root and capsule. *Macrophomina phaseolina* is pycnidial stage of *Rhizoctonia bataticola* and is only seen on living host plant and it forms

sclerotia on potato dextrose agar medium. To know about the disease incidence survey was carried out in major sesame growing districts of Rajasthan. Survey mainly based on topography and geography of soil, soil characterization like colour, texture and irrigation situation

Materials and Methods

Experimental site

The investigations were carried out during Kharif 2018 and Kharif 2019 crop seasons at the Agronomy Farm and Department of Plant Pathology, S.K.N. College of Agriculture, Sri Karan Narendra Agriculture University, Jobner, Jaipur Rajasthan. Jobner is situated at latitude 26˚5‟ N, longitude of 75˚20‟ E and altitude of 427 meters above MSL (mean sea level). The region falls under semi-arid eastern plain (Agro Climatic Zone- lll A) of Rajasthan.

Raising of crop

Cage house

For pot experiments, the plants were raised in 30 cm diameter earthen pots, pre sterilized with 2 per cent formalin solution and filled with sterilized soil in 3:1 (Soil : FYM) ratio which was autoclaved at 1.045 kg/cm^2 for 1 hour on 3 consecutive days. In each pot, eighteen healthy seeds of sesame (var. VRI-1) were sown and 20 seedlings were maintained in each pot.

Field experiments

The field was prepared before sowing by cross ploughing with tractor drawn disc harrow and planked. In all the field experiments, susceptible sesame variety (VRI-1) was used. The layout plans of different field experiments were demarcated in the field for treatment application. After applying the treatments, the crop was sown in the first week of July in both the years. The experiments were laid out in randomized block design (RBD) with required replications. The crop was raised in plots of 2 m x 2.1 m area, keeping row to row and plant-to-plant distance of 30 cm x 10 cm. The irrigation was applied as per the recommendation for the crop in this zone and one weeding and hoeing was done at 30 days after sowing. In all the experiments, inoculum multiplied on sterilized sorghum grains were applied in furrows at the rate of 20 g/m row length at time of sowing.

Survey and incidence of disease

Survey in major sesame growing districts of Rajasthan *viz*., Pali, Jodhpur, Nagaur, Tonk and Jaipur was undertaken during last week of September to first week of October to know the incidence of stem and root rot disease. Surveys were conducted during the Kharif season of 2017 in one hundred fields of twenty villages of ten tehsil of five districts. The selection of two villages from each tehsil was made randomly. To assess the disease incidence, five sesame fields were selected in each village in each tehsil of district and average incidence of the disease in each village was calculated. In each field, five spots of one square meter area were marked diagonally at randomly to cover entire field. Diseased and healthy plants were counted in each spot and the per cent disease incidence was calculated as per formula given below.

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

Collection, isolation and purification of pathogen

Stem and root rot affected plants of sesame were collected from surveyed areas of Rajasthan viz., Pali, Jodhpur, Nagaur, Tonk and Jaipur districts. The samples of diseased plants were used for isolation of the causal fungus. Isolation was done from the diseased stem and roots of sesame samples, collected during survey from the fields. The affected parts were thoroughly washed with tap water to remove soil. Small pieces of about 0.5 cm length were cut from stem and roots and surface sterilized with 1 per cent sodium hypochlorite solution for 1 minute followed by three washings with sterilized distilled water. The surface sterilized pieces were transferred aseptically on potato dextrose agar (PDA) slants in a Laminar Air Flow Cabinet and incubated at 25±1 °C temperature in a B.O.D. incubator for 7 days. To obtain pure culture of *Macrophomina phaseolina*, single hyphal tip isolation technique was adopted (Rangaswami and Mahadevan, 2004) [18]. One ml of suspension having 5-6 pieces of hypha per 10X microscopic field were spread over 2 per cent plain agar in Petri dishes evenly by tilting the Petri dishes clockwise as well as anti clock wise direction. The excess amount of suspension was decanted and Petri dishes were incubated at 25±1 °C for 24 hours in B.O.D. incubator. Single piece of hypha was demarcated under low power objective of microscope (10X) and cut with the help of dummy objective. Individual piece of hypha was transferred aseptically on PDA slants with the help of an inoculating needle. The inoculated slants were kept in B.O.D. incubator at 25±1 °C temperature. Purified cultures of five samples were tentatively identified as *Macrophomina phaseolina* on the basis of morphological and colony characters. Thus, ten isolates were established from each district and coded as MPpa1, MPpa2, MPjd1, MPjd2, MPjp1, MPjp2, MPng1, MPng2, MPtn1 and MPtn2. The purified cultures were maintained by periodical transfers on PDA slants and used for further studies.

Identification of the pathogen

The isolated fungus was identified on the basis of morphological and colony characters viz., white to grey colony, turning black with age, septate hyphae and blackish colour spherical, irregular and oblong type sclerotia as *Macrophomina phaseolina*. The fungus was identified as *Macrophomina phaseolina* based on unique morphological and cultural characteristics formation was seen. Round shaped, irregular and oblong type sclerotial formation was seen.

Multiplication of inoculum for inoculation

In the present study, the sorghum grains were used for multiplying inoculum (mycelia of *Macrophomina phaseolina*) for inoculations and other studies. The sorghum grains were soaked overnight in ordinary water. The excess water was drained out. About 150 grams of soaked sorghum grains and 30 ml of water were taken in each 250 ml conical flask, plugged with cotton and sterilized in autoclave at 1.045 kg/cm^2 pressure for 30 minutes. The substrate in flasks was inoculated aseptically with 7 day old mycelial discs (5 mm) of the pathogen/isolates and incubated for 20 days at 25 ± 1 °C.

Pathogenicity test

For proving pathogenicity, isolated and purified *Macrophomina phaseolina* was multiplied on sterilized sorghum grains and proved pathogenicity through seed, soil and soil cum soil inoculation technique. Soil collected from the field was autoclaved at 1.05 kg/cm^2 pressure for 30 minutes for three consecutive days. The inoculum multiplied on sorghum grains was added in the earthen pots (30 cm diameter) @ rate of 20 g/pot. The pots were surface sterilized

by 2 per cent formalin solution before filling the soil inoculum mixture. Eighteen apparently healthy sesame seeds of susceptible variety VRI- 1 were surface sterilized with 1 per cent sodium hypochlorite solution were sown in each pot with four replications. The pots without inoculum were served as control. Observations on disease symptoms were recorded on 60 days. Plants showing root rot symptoms were collected and the pathogen was re-isolated and compared with the original culture (Radha krishanan and Sen, 1985).

Seed inoculation technique

For this, seeds were rolled on 7 days old culture of fungus thiriving on PDA contained in Petri plates. The inoculated seeds were sown in cemented pots. The un –inoculated apparently healthy seeds served as check. These pots were kept in cage house and watered regularly as and when required.

Soil inoculation technique

Prior to sowing, pots (30 cm diameter) were sterilized with copper sulphate solution and filled with sterilized soil + FYM (Soil : FYM=3:1; sterilized at 1.045 kg/cm^2 for one hour for three consecutive days). These pots were inoculated with inoculum, multiplied on sorghum grains @ 20 g/pot. Healthy and surface sterilized sesame seeds (VRI-1) were sown in each pot with four replications. Surface sterilized seeds sown in uninoculated sterilized soil, served as check. These pots were kept in cage house and watered regularly as and when required and maintained under identical conditions.

Seed cum soil inoculation technique

In this technique above both techniques were carried out together and inoculum of pathogen was applied in soil and seed were also rolled with 7 days old culture of fungus and surface sterilized seeds sown in uninoculated sterilized soil, served as check.

Results and Discussion

Survey and occurrence of stem and root rot incidence in sesame

A roving field surveys of five major sesame cultivating districts i.e. Jodhpur, Nagaur, Pali, Jaipur and Tonk were performed during the Kharif season of 2017. The survey in these hotspots areas for stem and root rot disease was conducted during peak stage for disease development (between 40-60 days after sowing). A total one hundred fields from twenty villages of ten tehsil of these five aforesaid districts were surveyed and isolates of disease were collected along with information of such cultivar which were grown by the farmers, soil type, irrigation facility (rainfed or irrigated), disease incidence etc. The results of field survey presented in Table 1 revealed that that root rot incidence was varied from 23.84 - 53.72 per cent in five districts of Rajasthan. Highest mean disease incidence was observed in Jodhpur district (41.14%) followed by Pali (38.71%), Jaipur (35.90%), Nagaur (28.58%) and lowest was in Tonk district (24.41%). The disease incidence also varied among the tehsil and followed the sequence of decreasing order as Bilada (53.72%) > Mandor (48.57%) > Desuri (40.5%) > Kisangarh Renwal (37.79%) > Sumerpur (37.37%) > Phulera (34.01%) > Molasar (29.87%) > Degana (27.29%) > Malpura (24.98%) > Deoli (23.84%). The all over mean sesame stem and root rot disease incidence of these five surveyed districts was 33.74 per cent. Singh *et al*. (1991) [13, 21] and Choudhary *et al.* (2005) [6] done survey of sesame growing areas of North Bihar and recorded the stem and root rot incidence ranging from 22.5 to 38.5 per cent. Min and Toyota (2019) [16] also observed disease incidence from 10 to 30 per cent in sesame at Nay Pyi Taw and Myanmar. The survey revealed that when the crop sown under rainfed conditions showed more stem and root rot incidence (27.29 to 53.72%) as compared to crops sown under irrigated conditions (23.84 – 24.98%). Balabaskar *et al.* (2015) [4] and Thirunarayanan *et al.* (2017) [23] conducted surveys and also reported that dry conditions are more prevalent in the rainfed conditions and caused more damage because number of sclerotia increased under low rainfall conditions. The sandy loam had more stem and root rot incidence (27.29 – 53.72%) than clay (24.98%) and clay loam (23.84%). Low soil moisture have more aeration, so that development of mycelium increases. Sesame plants are highly susceptible to flooding and caused mortality into the plants. Therefore, irrigation has effect on for management of stem and root rot. The activity of the pathogen depends on available free oxygen in soil. *Macrophomina phaseolina* survives as seed and soil borne pathogen and competition occurred between plants and microorganisms at germination stage. Sandy soils have more number of macropores and poor in water holding capacity (Baver *et al.*, 1962) [5]. Similarly, Rettinasababady and Ramdoss (1999) [19], Cruz Jimenez (2011) [7] and Karibasappa *et al*. (2018) [10] showed that maximum stem and root rot incidence found under sandy soils, followed by loamy sand and loam soil textures. The occurrence and severity of root rot incidence was directly related to the availability of sclerotia present in the soil. (Khan, 2007) [12].

Table 1: Survey and occurrence of stem and root rot incidence in sesame in different locations in Rajasthan

Isolate no.	District	Tehsil	Village	Situation	Cultivar	Soil type	Disease incidence (%)
MPp1	Pali	Sumerpur	Angor	Rainfed	Local	Sandy loam	37.37
MPp2	Pali	Desuri	Sadari	Rainfed	Local	Sandy loam	40.05
							Mean 38.71
MPjd1	Jodhpur	Bilada	Bilada	Rainfed	Local	Sandy loam	53.72
MPjd2	Jodhpur	Mandor	Mandor	Rainfed	Local	Sandy loam	48.57
							Mean 41.14
MPng1	Nagaur	Molasar	Molasar	Rainfed	Local	Sandy loam	29.87
MPng2	Nagaur	Degana	Degana	Rainfed	Local	Sandy loam	27.29
							Mean 28.58
MPtn1	Tonk	Malpura	Bagri	Irrigated	Local	Clay	24.98
MPtn2	Tonk	Deoli	Beejwar	Irrigated	Local	Clay loam	23.84
							Mean 24.41
MPjp9	Jaipur	Phulera	Basingpura	Rainfed	Local	Sandy loam	34.01
MPjp10	Jaipur	K.renwal	Hathipura	Rainfed	Local	Sandy loam	37.79
							Mean 35.90
							Over all mean= 33.74

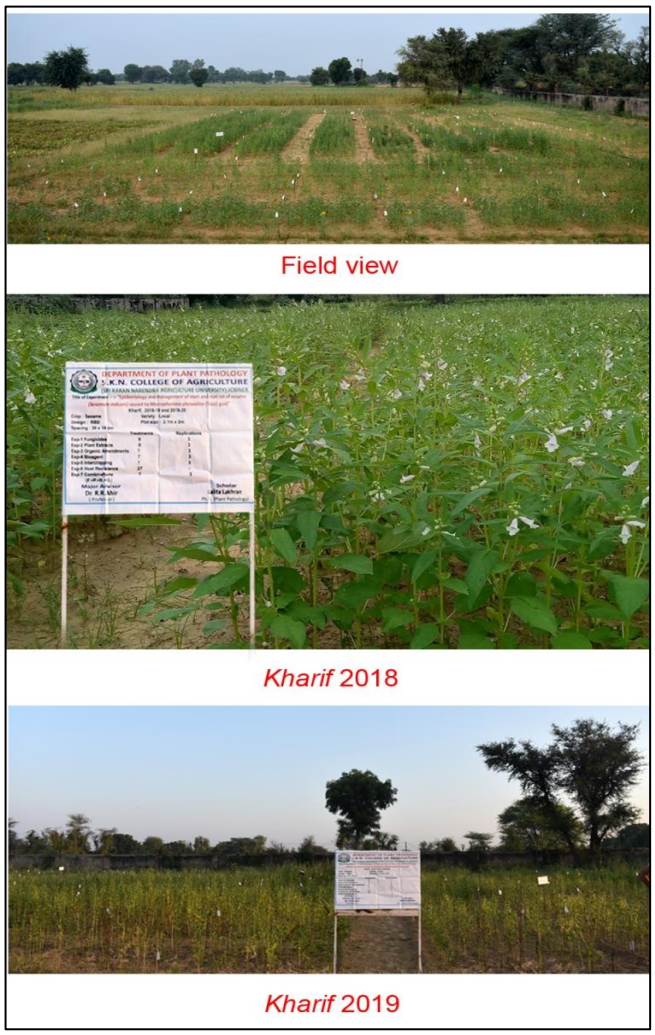

Plate 1: field trials during Kharif 2018 and 2019

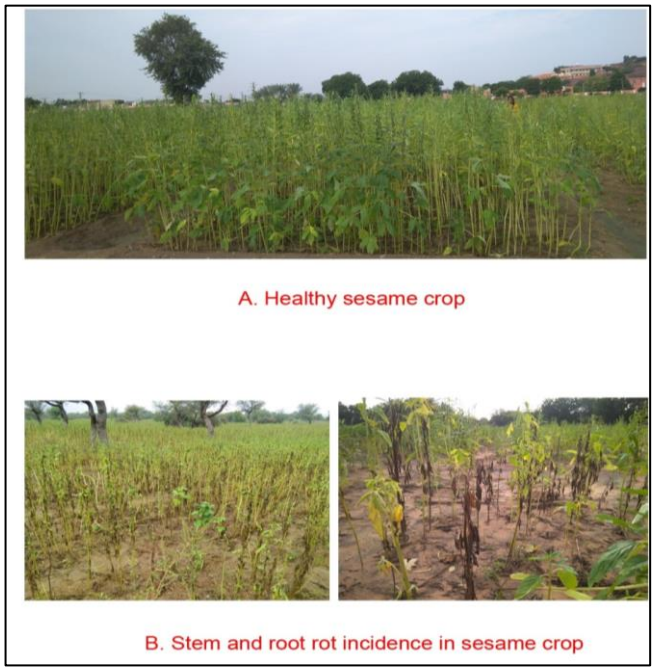

Plate 2: Field Survey of sesame crop During kharif 2017

Collection, isolation, purification and identification

Disease infected plants of sesame were collected from all the five surveyed districts *i.e.* Jodhpur, Pali, Jaipur, Tonk and Nagaur which considered as hotspots for root rot incidence. Infected stems and roots of plants which were collected from different locations were placed on Potato Dextrose Medium under aseptic condition. After isolation of fungus on PDA plates were incubated and yielded profuse fungus growth after seven days of inoculation. A whitish mycelial growth appeared on PDA which later turned into brownish black when culture become old. After 10-15 days fungus developed black hard sclerotia in the peripheral areas of the colony. The fungus was purified with the help of hyphal tip cut method.

Identification of the pathogen

The visible morphological and cultural characteristics of sclerotia served as primary tool for identification of the isolated fungus. Emerging hyphae of fungus having hyaline which profused whitish in colour later it was becomes brownish black in colour with abundant septation. Profuse secondary hyphae formed in a set pattern of branching from the right angle of parental hyphae. Round shaped, irregular and oblong type sclerotial varied in size from 70.20- 106.50 µm were formed. The sclerotia of the fungus were light brown in the starting phase which turned brown to black in final stage of growth. Rough, irregular, beaked and ostiolated type pycnidia of fungus were found on surface of host which were dark brownish to blackish colour and bigger in size than sclerotia. Therefore, based on these observed morphological and cultural characteristics, it was identified as *Macrophomina phaseolina* (a pycnidial stage of *Rhizoctonia bataticola)* with ID No. 6621. The similar results were finding by Mehta (1951) [15] First report of root rot pathogen in sesame caused by *Macrophomina phaseolina* from Uttar Pradesh and Dhingra and Sinclair (1977) [8] in pulses and oil seed crops. Sun *et al.* (2015) [22] isolated four isolates of adzuki bean and identified as *M. phaseolina* based on morphological and molecular characteristics.

Pathogenicity of *Macrophomina phaseolina*

A pot experiment was conducted to prove the pathogenicity of the isolated fungus. The fungus was found pathogenic and the typical disease symptoms appeared on stem and root parts of the infected plants within 30-40 days after sowing time. The first sign of disease was sudden wilting of sesame seedlings. The infected leaves become yellowish and started to drop out within two- three week and blackish coloured sclerotia developed on stem and within roots. Finally, profuse growth of fungus in the root system resulted in complete blockage of the vascular system of root and consequences of this blockage roots were decayed from ground surface. The result of pathogenicity test against root rot pathogen revealed that the highest disease incidence (53.84%) was observed under seed cum soil inoculation followed by soil inoculation technique (46.66%) (Table 2). Likewise, minimum seed germination (40 days after sowing) (72.22%) was observed with seed cum soil inoculation followed by soil inoculation (83.33%) and maximum seed germination was in uninoculated control (94.45%). These studies were supported by Khamari *et al.* (2017) [11] and found that the soil inoculated with *Macrophomina* + *Fusarium* and *Macrophomina* alone gave 34% seed germination and in control pot recorded 82% germination. The first sign of disease appear sudden wilting of sesame seedling. The infected leaves become yellowish and started to drop out within two- three week and blackish coloured sclerotia developed on stem and within roots. Finally, profuse growth of fungus in the root system resulted in complete blockage of the vascular system of roots and

consequences of this blockage, roots rotted out from ground level. This experiment is conformity of earlier findings by John (2000) [9] who observed occurrence of root rot in sesame field and characterized pathogen on culture media having hyaline and septate mycelium and branching pattern was right angle with a constriction at the origin point. Mycelium was initially whitish in colour which later turned grayish brown to blackish in colour and formed black coloured sclerotia on PDA medium. Same results were found by Jakhar (1997) and proved pathogenicity test through Koch's postulates techniques. El–Araby *et al*. (2009), Rangaswami and Mahadevan (2004) [18] observed disease symptoms including shrunken, brown wilted stem and roots due to *Macrophomina phaseolina* in soybean. Akhtar *et al*. (2011) [1] found necrotic behaviour of pathogen in sesame with seed infection efficiency up to 100 per cent.

Table 2: Pathogenicity test of *Macrophomina phaseolina* in sesame

Inoculation technique	Seed germination (%)*	Per cent disease incidence
Soil inoculation	83.33	46.66
	(58.19)	
Seed inoculation	94.45	41.17
	(65.90)	
Seed + Soil inoculation	72.22	53.84
	(48.19)	
Uninoculated control	94.45	0.00
	(76.37)	
SEm±	1.13	
CD (p = 0.05)	3.48	

Plate 3: Pathogencity test of macrophomina Phaseolina

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