

Phytopathogenomics and Disease Control., 2024, 3(2)261-266

ISSN (Online):2957-5842; ISSN (Print):2957-5834

DOI: https://doi.org/10.22194/Pdc/3.1037 https://societyfia.org/journal/PDC



Management of Fusarium Wilt Disease in Sesame (Sesamum indicum) through Seed Treatment and Soil Amendment

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Sesame holds a dominant position in global agriculture, primarily due to its rich protein content. This crop, however, faces a considerable threat in the form of Fusarium wilt, caused by the destructive Fusarium oxysporum. Despite advancements in agricultural technology, a significant portion of worldwide sesame production continues to be marred by this devastating disease. To address this issue, the current study was designed to manage Fusarium wilt in sesame through a series of screening trials involving various varieties, soil amendments, and seed treatments using different chemicals. The screening trial was carried out at the Ayub Agricultural Research Institute; with disease severity recorded using the rating scale. Among the six varieties/lines assessed, two varieties (Black Till and Till-18) exhibited resistance to the disease. To combat Fusarium wilt, the efficacy of different fungicides at varying concentrations was evaluated via petri plate culture assays in laboratory conditions. Among the three fungicides tested (Topsin-M, Carbendazim, Metallaxyl+Mancozeb), Carbendazim displayed the highest disease inhibition at all concentrations, surpassing Topsin-M and Sherit. Notably, the 200 ppm concentration of Carbendazim exhibited a disease incidence of 55.74%, while the 300 ppm and 400 ppm concentrations yielded 56.13% and 56.53%, respectively. Similarly, the effectiveness of various soil amendments (Poultry manure, Rice husk, Sugarcane bagasse) at different concentrations were assessed against the test pathogen under field conditions. Among these amendments, Poultry manure exhibited the highest disease inhibition at all concentrations compared to sugarcane bagasse and rice husk. The 10% concentration of Poultry manure resulted in a disease incidence of 58.21%, whereas the 20% and 30% concentrations yielded 56.87% and 59.54%, respectively.

Keywords: Fusarium Wilt, Sesame, Fungicide Efficacy, Soil Amendments, Disease Management.

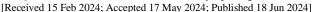
INTRODUCTION

Sesame (*Sesamum indicum* L.) belongs to the family Pedaliaceae (Noorka *et al.*, 2011). Sesame is acknowledged, like other early crop species in arid and semi-arid parts of the globe, as a rich oil source (Bedawy and Moharam, 2018). It is an important oilseed crop, grown for its seeds, which contain high-quality oil and proteins. The crop is widely cultivated in many parts of the world, including Asia, Africa, and Latin America. Sesame is one of the oldest oilseed crops in the world and has been cultivated for thousands of years. It is believed to have originated in Africa and was then introduced to Asia and the Middle East, where it has been an important crop ever since. The ancient Egyptians are known to have used sesame seeds as a medicine and as a food source, and

they were also used in religious ceremonies. Sesame seeds were also highly valued in ancient Babylon and Assyria, and were used as a form of currency. In Japan, sesame seeds were used in traditional dishes such as gomaae and tahini. In Asia, sesame seeds have been an important ingredient in cooking and traditional medicine for thousands of years (Daita *et al.*, 2018).

Sesame owing to the presence of large amounts of fatty acids, with the exception of high protein levels (22.0%), is considered the queen of oilseed crops. Sesame has been shown to contain 23.5% proteins, 13.5% starch, and 5% tar (Akintunde *et al.*, 2004). Furthermore, it is regarded as a rich supplier of numerous other nutritional components, including iron, magnesium, manganese, copper, and calcium. Sesame oil has 47% polyunsaturated fats and 39% lenolinic acid

Tahir, M.N., A. Habib, G.M. Sahi, M. Sagheer, T. Murad, K. Zahid and A. Ali. 2024. Management of Fusarium wilt disease in Sesame (*Sesamum indicum*) through seed treatment and soil amendment. Phytopathogenomics and Disease Control 3:261-266.





(Shyu *et al.*, 2002). Its oil content varies from 50-58%. Sesame seeds are widely used in sweets and pastry items, as well as in manufacturing soaps, perfumes, vegetable oils, and carbon fibre too. India, Sudan, China, and Burma contributed 60% of the whole output. Sesame seeds are used in a variety of cuisines and products, including tahini, halva, hummus, and sesame oil. (El Khier *et al.*, 2008).

In 2020, roughly 6.8 million tons of sesame seeds were collected globally, which were cultivated on an area of 13.97 million ha (FAO, 2020). As per the National Bureau of Statistics for the year 2018-19, consumption of Pakistan stands at 366 million tons, equating to PRs. 9,000 million. About 29 thousand tons of sesame seeds were harvested over an area of 176.0 thousand acres in Pakistan. In Pakistan, it is grown in various parts of the country, including Sindh, Punjab, Khyber Pakhtunkhwa, and Balochistan. The crop is typically sown in April and harvested in September or October. Suitable places for sesame farming in Pakistan include districts of Gujranwala, Attock, Bhakar, Gujrat, Sialkot and Faisalabad in Punjab; Sadu, Tharparkar and Hyderabad in Sindh; Kohat and D.I. Khan in Khyber Pakhtunkhwa, and Naseerabad and Lesbela in Baluchistan. Pakistan exports sesame seeds to various countries, including Japan, South Korea, China, Turkey, and the Middle East. The exports of sesame seeds during the fiscal year 2020-2021 were around 27,140 metric tons, with a value of around PKR 3.3 billion (approximately USD 21 million). Sesame crop contributes significantly to the agricultural economy of Pakistan. The total value of sesame production during the fiscal year 2020-2021 was estimated to be around PKR 4.2 billion (approximately USD 27 million). The crop is an important source of income for small farmers and rural communities, and it is used both for domestic consumption and for export (Singh et al., 2016).

In addition to the biologically projected poor-producing crop, several pathogenic and climatic influences are adding to its low productivity. Fusarium wilt disease is a serious fungal disease that can affect sesame plants. It is caused by the soilborne fungus Fusarium oxysporum f.sp. sesami, which can survive in the soil for long periods of time. The fungus infects the sesame plant through the roots and then spreads throughout the plant's vascular system, causing damage to the plant's ability to transport water and nutrients. Fusarium wilt (Fusarium oxysporum f. sp. sesame) its incidence range 17.1 to 73.3%. The considerably more dangerous issue is "sesame droop" induced by the well-established soil-borne fungus (Fusarium oxysporum f.sp. sesame) causing substantial reduction in the sesame yield (Ngamba et al., 2020). Many techniques have been tried to decrease the Fusarium wilt inflicting damages on sesame crops including drought resistance, crop rotation, fumigation, etc. While the deployment of different resistant plants is a proven, feasible strategy to manage the wilt problem, whereas the recurrence of new virulent strains and subsequent broke down of host defense system is a considerable issue (Kebede, A. A., and H. Tsehaye. 2020).

MATERIALS AND METHODS

Collection of Diseased Samples: Diseased samples of sesame were collected from different fields of Faisalabad. These samples were transferred to the Seed Health Testing Laboratory, University of Agriculture Faisalabad.

Isolation, Purification and Identification of F. oxysporum: For isolation of *F. oxysporum*, the small portion from infected sample of sesame was collected and transferred to the PDA medium in Petri dishes and incubated in dark at 25°C for 6 days. A small portion of the fastest growing colony of *F. oxysporum* was taken from the periphery of 90 mm diameter Petri dish and spread on Petri dishes containing PDA and incubated in the dark at 25°C for 5 days. Morphological characteristics were studied in order to identify the fungus. Moreover, characteristics spores of the fungus were analyzed under microscope.

Confirmation of Pathogenicity: Studies were carried out to confirm the Pathogenicity of the isolated pathogenic fungus. Soil was sterilized by mixing it thoroughly with formaldehyde solution at the rate of (100 Ml/kg) of the soil. Proper humidity level was maintained by covering the sterilized soil with wet gunny bags for two days. The soil was inoculated with a pure culture of the test pathogen by embedding discs of PDA containing the pathogen's mycelial mates at a certain depth. It was then covered with a layer of soil, periodically watered, and left undisturbed for five days. Eight pots were utilized for this experiment, while control pots contained solely PDA discs. Subsequently, sesame seeds were subjected to surface sterilization using 2% sodium hypochlorite for duration of two minutes. These seeds were sown in pots when inoculum was spread vigorously. Pots were placed under lab conditions and irrigated with sterilized water during the growth phases. Data regarding to the plant infection was recorded on germination after 14 days.

Screening of Sesame varieties/lines against Fusarium wilt: Seeds of Sesame were sown in field under Randomized Complete Block Design (RCBD). In field plant to plant distance 10-12 cm and row to row distance 45 cm was maintained with three replications. Length of each row was 10 feet. Field plots were inoculated with *F. oxysporum* per meter of row. Data was recorded by using disease rating scale (Dinkaran *et al.*, 1996) of given in table

List of varieties:

V1	=	Black Till
V2	=	Til- 18
V3	=	Punjab Till-89
V4	=	TS-3
V5	=	TS-5
V5	=	TH-6
V6	=	TH-6



Disease Rating Scale for screening trials: Data of screening was recorded by using following rating scale (Prasad *et al.*, 2021).

Score	Description	Disease Reaction
0	Free from The Disease	Immune (I)
1	No Symptoms on the Plant	Resistant (R)
3	10 % or less mortality	Moderately Resistant
		(MR)
5	11-20% mortality	Moderately Susceptible
		(MS)
7	20-50% mortality	Susceptible (S)
9	51 % or more Mortality	Highly Susceptible
		(HS)

Inoculation of Sesame Seeds: Sesame seeds were intentionally contaminated with a test pathogen by immersing them in a suspension containing F. oxysporum. To achieve this, 10 ml of sterilized water was introduced into the F. oxysporum culture plate and agitated with a finger to dislodge the fungal growth from the medium. The seeds were immersed in this suspension for duration of 12 hours. Subsequently, the seeds were air-dried on blotter paper before being planted in the field.

Seed Dressing by Fungicides: Description of fungicides used is as following:

-	1	Topsin-M	Thiophenate	70 WP	Arysta Life science
			Methyl		
2	2	Shinkar	Carbendazim	50 WP	ICI Pakistan Ltd.
(3	Sherit	Metallaxyl +	72 WP	Star Agro Sciences
			Mancozeb		(Pvt.) Ltd.

Inoculated seeds were dressed with 200ppm, 300ppm, and 400ppm of fungicides and placed on PDA poured petri plates. These were incubated at 25 °C and growth was observed after 24hr, 48hr and 72 hr.

Evaluation of soil amendments against Fusarium wilt: Soil amendments such as sugar cane bagasse, rice husk, and poultry manure were added by following concentrations i.e. 10%, 20% and 30%.

Data Recording: Disease incidence (DI) was recorded after 30 days of seed germination by using following formula

Disease incidence (%) =
$$\frac{(No.of\ infected\ plants)}{Total\ plants} \times 100$$

(Ali et al., 2010)

RESULTS AND DISCUSSION

Frequency of various fungi associated with infected sesame samples: Analysis of fungal colonization in infected sesame samples revealed varying frequencies. F. oxysporum exhibited the highest colonization, reaching 73%, after a 7-day incubation period, surpassing A. niger (53.3%) and A. flavus (33.3%) across all Petri plates. Among these fungi, F. oxysporum emerged as the dominant species, followed by A. niger and A. flavus. For further study, pure colonies of F. oxysporum were carefully isolated and transferred to other

Petri plates to facilitate pathogen multiplication. This isolation process was carried out under aseptic conditions to maintain the integrity of the samples. The isolation process successfully identified *F. oxysporum*, *Aspergillus niger*, and *Aspergillus flavus* in the collected samples. These results align with those reported by Nik *et al.*, (1983), who identified Fusarium spp., *Fusarium oxysporum*, and *Pestalotiopsis* spp. as pathogenic fungi in samples of *Vigna radiata* from various localities. Similarly, Rashid *et al.*, (1983) obtained similar findings when they isolated *Colletotrichum dematium*, *Alternaria alternata*, *Fusarium oxysporum* and *F. semitectum*, from fifteen seeds of five different verities of *Vigna radiata*.

Screening of sesame cultivars against Fusarium wilt disease caused by Fusarium oxysporum: This study observed that different sesame genotypes exhibited varying levels of resistance or susceptibility to F. oxysporum when grown in field conditions. Out of six varieties/lines two varieties (Black Till, Till-18) showed resistant behavior. Two lines (TS-5, TH-6) showed moderately resistant behavior, while one line (Punjab-Till-89) showed susceptibility and one line (TS-3) showed highly susceptible towards F. oxysporum as shown in Table.1. F. oxysporum poses a significant threat to the Fabaceae family in Pakistan, leading to potential yield losses of up to 100% during epidemics. Employing host plant resistance stands out as the most practical and cost-effective strategy for mitigating these losses. Prior to launching breeding programs focused on developing resistant varieties, it is crucial to identify sources of resistance.

The results we obtained are backed by the research of scientist Rajput *et al.* (1997), who evaluated 107 sesame genotypes in a field nursery during the Kharif season of 1997 for resistance against *Fusarium oxysporum*. Among these genotypes, one showed complete resistance and remained entirely free from infection. Forty-six genotypes exhibited a safe response, fifty-five showed moderate safety, and five genotypes displayed a moderately vulnerable response.

Table 1. Genetic response of different screened varieties/lines of sesame

varieties/fines of sesume			
Rating	Disease reaction	Varieties	
0	Immune (I)		
1	Resistant (R)	Black-Till, Till-18	
3	Moderately Resistant (MR)	TS-5, TH-6	
5	Moderate Susceptible (MS)		
7	Susceptible (S)	Punjab Till-89	
9	Highly Susceptible (H.S)	TS-3	

Our findings align closely with those of Dubey *et al.* (2009), who investigated Fusarium wilt disease resistance, in three sesame varieties (ORM 7, ORM 14, and ORM 17) alongside test varieties planted between June 2000 and May 2001. All three cultivars exhibited resistance to the disease. Similarly, Wang *et al.* (1991) conducted a study in China where they



identified three sesame varieties (S-17, PR-19-9, and Ts-3) as the most resistant against root rot and head rot diseases in sesame.

Evaluation of different seed treating fungicides against F. oxysporum: The graphical presentation (Fig.1.) revealed highly significant results for all seed treatments, concentrations, and their interaction. Specifically, the and interaction between treatments concentrations demonstrated that Carbendazim achieved the highest disease inhibition across all concentrations compared to other treatments such as Sherit and Topsin M. At a concentration of 200 ppm, Carbendazim resulted in a disease incidence of 55.74%, while at concentrations of 300 ppm and 400ppm; it showed disease incidences of 56.13% and 56.53% respectively. Similarly, Sherit demonstrated inhibition percentages of 52.01%, 52.57%, and 53.14% at concentrations of 200 ppm, 300 ppm, and 450 ppm respectively. Meanwhile, Topsin M exhibited disease inhibition percentages of 45.62%, 46.06%, and 46.51% at concentrations of 200 ppm, 300 ppm, and 400 ppm respectively.

Our findings are corroborated by the discovery made by Singh and Kaiser (1995), who noted that among the eight fungicides examined both in laboratory and field settings, Carbendazim exhibited the highest effectiveness against *F. oxysporum*. This observation aligns with the findings of Khan and Khan (2006), who reported that both benomyl and Carbendazim fully inhibited the mycelial growth of *F. oxysporum*. Similarly, Suryawanshi *et al.* (2008) arrived at comparable results, concluding that Carbendazim not only inhibited the growth but also the sclerotial production of *F. oxysporum*. Our results align with those of Kulkarni (2000), who evaluated various fungicides against safflower root rot caused

Our results align with those of Kulkarni (2000), who evaluated various fungicides against safflower root rot caused by *F. oxysporum* and concluded that carbendazim and propiconazole were the most effective fungicides.

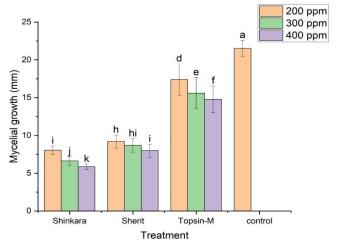


Figure 1. Graphical view of different seed treating fungicides against Fusarium wilt.

Evaluation of soil amendments against F. oxysporum: Different soil amendments materials were used on sesame under vivo conditions in pots to check their control against fusarium wilt. Soil amendments such as sugar cane bagasse, rice husk, and poultry manure were added by following concentrations i.e. 10%, 20% and 30%.

Table 2. Comparative analysis of different soil amendments against Fusarium wilt.

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Sr#	Treatment	Disease incidence %	
1	Poultry Manure	24.73 e	
2	Rice Husk	27.21 d	
3	Sugarcane Bio	29.32 c	
5	Control	50.13 a	
LSD		6.53	

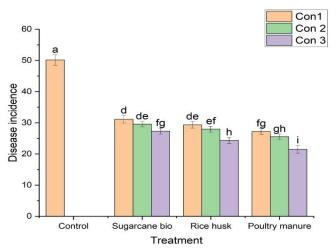


Figure 2. Graphical view of different soil amendments against Fusarium Wilt disease of sesame.

The above given table reveals highly significant results for all treatments, concentrations, and their interaction (Table 2). Specifically, the interaction between treatments and concentrations demonstrates that poultry manure displayed the highest level of disease inhibition across all concentrations, surpassing other treatments such as sugarcane bagasse and rice husk.

The concentration 10% of Poultry manure 58.21% disease incidence as compared to 20% (56.87) and 30% (59.54). Similarly, rice husk showed (55.48, 55.84, 56.3) percent disease inhibition at 10%, 20% and 30% concentrations respectively. Our findings related to soil amendments have contradict with previous research studies including Rao *et al.*, 2022, were out of nine organic amendments tested against Fusarium wilt in pot conditions, neem seed kernel cake recorded the lowest wilt incidence, followed by mustard cake and poultry manure and in line with findings of Barakat and Al-Masri, 2009.



Conclusion: In case of soil borne pathogens like Fusarium, depending on resistant lines for successful sesame cultivation not effective. So applying some other integrated approaches including different seed treatment chemicals and some organic soil amendments as solo or in consortium could be better options for maximum crop production targets.

Author's Contribution: M. Nouman Tahir and T. Murad: Conceptualization, conducting the field trials and writing original draft.

A. Habib: Outline and finalization the review.

G. M. Sahi and Muhammad Sagheer: Visualization, collecting literature, validation and editing.

Khizra Zahid and Ansar Ali: Experimental layout plan and statistical analysis.

Conflict of interest statement: The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical statement: This article does not contain any studies regarding human or animal

Data availability statement: All the data presented in this article newly recorded and analyzed statistically and will be provided if needed at any stage.

Acknowledgement: Not applicable.

Funding: Not applicable.

Code availability: Not applicable.

Consent to participate: All authors participated in this research study.

Consent for publication: All authors' submitted consent to publish this research work.

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