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Management of *Macrophomina phaseolina* Infecting Sesame Germplasm

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ABSTRACT

Introduction: Sesame (*Sesamum indicum* L.) is an important oilseed crop, grown worldwide as a rich source of premium quality edible oil. Charcoal rot of sesame is a major disease that causes drastic yield losses. This research was performed to access the management of *Macrophomina phaseolina*, cause of charcoal rot. **Methods:** The study involved evaluation of ten sesame genotypes to study their reaction towards charcoal rot disease. Fungicides were evaluated under laboratory and field conditions. The field experiments were carried out under randomized complete block (RCB) design whereas the laboratory experiment with completely randomized (CR) design. **Results:** Seven sesame lines exhibited moderate resistance to charcoal rot disease while three lines were moderately susceptible with 21-30% severity. Among fungicides, Nativo exhibited highest growth inhibition of *M. phaseolina*. **Discussion:** The results demonstrated that the resistance level of each sesame genotype and the most effective fungicide to manage the charcoal rot disease.

Keywords: *Sesamum indicum*, Charcoal rot, resistance, Nativo.

INTRODUCTION

Sesame (*Sesamum indicum* L.), also referred to as Till or Gingelly, belongs to Pedaliaceae family and the Sesamum genus. It is thought to be the first oilseed crop known to human beings (Ram *et al.,* 1990). The wild relatives of sesame are mainly found in sub-Sahara in Africa (Bedigian, 2010), but a few are also found in India (Baydar, 2005). Sesame's archeological evidence dates to 2250 and 1750 BC in Harappa, Pakistan. The Sesamum genus has undergone significant development and differentiation in Africa, with around identified 30 species (Arslan et al., 2007). Sesame can be cultivated in various types of soil, it thrives best in soils with good drainage, fertility, and neutral to slight alkalinity having pH range of 5 to 8.

The sesame seeds are highly enriched with edible oil and various nutrients that are highly beneficial to health due their nutritional and medicinal properties (Ghias *et al.*, 2021). The seeds contain 55% oil content and is ranked 1st among all major oilseed crops (Farooq *et al.*, 2019). The oil

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contains linoleic acid (39%) and oleic acid (47%) that make it premium in quality (Akbar *et al.,* 2012).Various sesame accessions contain high levels of sesamin (0.67-6.35 mg per gram) and gamma tocopherol (56.9-99.3 µg per gram), which have been linked to the prevention from hypertension and stroke (Pusadkar *et al.,* 2015). Globally, it is grown on an area of about 14 million ha with an average yield of 4872 kg (12.18 mounds) per hectare (FAOSTAT, 2020)..

Charcoal rot is caused by *Macrophomina phaseolina* Goid (Tassi), a soil- and seed-borne fungus. Generally, it can cause up to 30% yield losses or even complete loss of sesame crop in severe cases. Among 100 plant families, the pathogen can infect almost 500 different species (Gomez-Cadena *et al.*, 2016). The charcoal rot mostly occurs at the onset of flowering and becomes severe at crop maturity. The disease mainly occurs during hot dry weather with black colored fungal spots formed on root or stem (Wang Linhai, 2011). The main origin of contamination from *M. phaseolina* lies in microsclerotia, a resilient structure that has the capability to subsist for up to fifteen years in the soil. At the initial stage of seedling, the roots of the host plant can encounter the microsclerotia, which can invade them through multiple budding hyphae (Gupta *et al.,* 2012). Once the fungus infiltrates the roots, it has the ability to disrupt the xylem-phloem system, which can interfere with the transportation of essential nutrients and water to the upper parts of the plant.

There is a need for improved strategies to manage charcoal rot in a cost-effective manner, as current approaches are insufficient. However, the disease control is somewhat difficult due to variable nature of pathogen. The disease can be controlled either by developing resistant cultivars or applying chemical fungicides of different properties. Integrated disease management (IDM) consisting of resistant cultivars, appropriate sowing time, proper seed treatment, and pesticides application is an effective strategy to control the disease and improve the plant health (Liu *et al.*, 2021). The chemical control of charcoal rot disease by using different fungicides is an easy and immediate approach (Meena, 2020). Different fungicides are available to treat seed, soil, or plant foliage to combat this disease. The treatment of seed with an appropriate fungicide before sowing is a preventive way to minimize the attack of this pathogen. The most common fungicides for seed treatment include thiram, thiophanate-methyl, carboxin, mancozeb and captan (Wu *et al.,* 2014).

Keeping in view the current challenges, this study aimed at screening of existing sesame germplasm by evaluating their response towards charcoal rot disease and the management of disease using different fungicides.

MATERIALS AND METHODS

Collection, Isolation and Identification of *M. phaseolina*

The sesame plants showing symptoms of charcoal rot disease were collected from the research fields of the University of Agriculture Faisalabad and Oilseeds Research Institute, Ayub Agricultural Research Institute, Faisalabad. The infected stem bark of sesame plants containing the sclerotia of the *M. phaseolina* was procured and treated to isolate the pathogen. The affected plant stem tissues were excised and cut into small fragments, which were then cleaned with tap water to eliminate any residual dust The tissue pieces were surface sterilized with 1% sodium hypochlorite solution for 1-2 minutes followed by three rinses with sterile distilled water to eliminate residual bleach (Joshi *et al.*, 2006). Two to three tissue pieces were placed in each petri plate, sealed with parafilm and incubated at 25±2°C for 48-72 hours (Cano-Reinoso *et al.*, 2021). The antibiotic streptomycin sulfate suppresses bacterial contamination during isolation (Kariola *et al.*, 2003). The fungi were subsequently examined and identified under a microscope by considering the morphological characteristics, including mycelium and spores (Mahdy and Mahmoud, 2022).

Preparation of *M. phaseolina* Inoculum

The preparation of inoculum was carried out under aseptic conditions by cultivating the pathogen on sorghum seeds. The seeds were immersed in sterilized distilled water overnight, air-dried at room temperature, and placed in conical flasks for autoclaving. The flasks were sealed with cotton swabs and covered with aluminum foil before being subjected to autoclaving. The flasks were then incubated at 26°C for approximately 15 days to facilitate pathogen colonization (Iqbal *et al.,* 2010).

Evaluation of Sesame Genotypes for Resistance against *M. phaseolina* Causing Charcoal rot Disease

After preparing the inoculum, a field plot was established through the inoculation of the soil with *M. phaseolina* inoculum at a rate of 40g/m^2. The inoculum was finely mixed with the upper layer of soil. The formalin-sterilized sand was then disseminated over the soil and irrigated to facilitate the growth of the pathogen (Farooq *et al.*, 2019). The screening of sesame germplasm for evaluation of resistance against charcoal rot disease was subsequently conducted in the sick-field. The resistance levels against charcoal rot disease in sesame germplasm were studied in sick-field by growing them at ridges with row-row and plant-plant distances of 45 cm and 30 cm respectively. The evaluation was carried out in RCBD with five replications for each genotype. The data was collected by counting the diseased sesame plants of each genotype at all stages of crop growth. To calculate the percentage disease incidence, following formula was used (Ghias *et al.*, 2021):

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

Management of *M. phaseolina* Causing Charcoal Rot of Sesame

The management of *M. phaseolina* was carried out under both laboratory and field conditions to evaluate the efficacy of six fungicides viz. Nativo (Tebuconazole 50% + Trifloxystrobin 25%), Cabrio Top (Pyraclostrobin 12.8% + Metiram 55%), Topsin-M (Thiophanate methyl 70%), Ridomil Gold (Metalaxyl-M 4% + Mancozeb 64%), Captan (Captan 50%) and Shincar (Carbendazim 12% + Mancozeb 63%) for the inhibition of growth of pathogen.

In-vitro* Evaluation of Fungicides against *M. phaseolina

The efficacy of six fungicides was evaluated against *M. phaseolina* through the poisoned food technique. The stock solutions were prepared by dissolving the technical grade fungicides in sterile distilled water. The concentrations tested were 150, 250 and 350 ppm prepared by transferring appropriate aliquots from the stock solutions to molten PDA medium. About 20 ml of the poisoned PDA was dispensed into 9 cm diameter sterile petri plates inside a laminar flow chamber (Dhingra and Sinclair, 1978). The poisoned PDA plates were inoculated centrally with a 5 mm mycelial disc obtained from 7-day old culture of *M. phaseolina* and incubated at 28±2°C. For each treatment, three replicates were maintained. PDA without fungicides served as control. The colony growth or colony diameter (cm) was measured after 1-, 2-, and 3-days post inoculation along two perpendicular directions and the mean was calculated (Bashir *et al.*, 2017).

Evaluation of Fungicides against *M. phaseolina* Under Field Conditions

A sick field was developed to evaluate fungicide efficacy under natural disease pressure. The susceptible sesame cultivar TH-6 was sown with 45x15 cm spacing in small plots at the experimental site. The soil was sandy loam with a pH of 7.5. The crop was maintained under recommended agronomic practices (Bashir *et al.*, 2017). At 30 days after sowing, the foliar sprays of fungicides were carried out using a hand operated sprayer. Three liters of fungicide solution was applied per plot providing uniform coverage. The treatments comprised of six fungicides each applied at their most effective concentration (350 ppm) determined through the *in-vitro* study along with an untreated control. Disease assessment was done at 10 days interval until crop maturity by recording the number of infected plants showing characteristic symptoms of charcoal rot. The disease incidence percentage (DI %) was calculated using the formula:

DI (%) = Number of infected plants/total observed plants x 100

The experiments were repeated and the average of two year data were statistically analyzed using Statistix 8.1 software.

RESULTS

Evaluation of Sesame Genotypes for Resistance against *M. phaseolina* Causing Charcoal Rot Disease

The sesame lines with same homogenous groups were non-significantly different from one another in disease resistance potential. The highest disease incidence was observed in line 19002 (24.08%) whereas, the lowest disease incidence was observed in line 16005 (15.03%). Three lines 19002, TH-6, and 70004 with disease incidence 24.08%, 23.6%, and 23.14% respectively were also non-significantly different from each other. Similarly, non-significant differences were found in 20001 (17.91%), 16003 (17.46%), 19003 (16.90%), 18002 (16.89%), and 18003 (15.34%) sesame lines. However, NIAB-M (19.15%) was significantly different form 16005 line (15.03%) and other sesame lines. The mean value of each line is shown graphically in figure 1.

Based on mean values of DI (%), the sesame lines were divided into two groups viz. moderately resistant and moderately susceptible. Seven lines (NIAB-M, 20001, 16003, 19003, 18002, 18003, and 16005) were classified as moderately resistant lines. Whereas the remaining three lines (19002, TH-6, and 70004) were grouped as moderately susceptible lines. No line was found completely resistant to Charcoal rot of sesame.

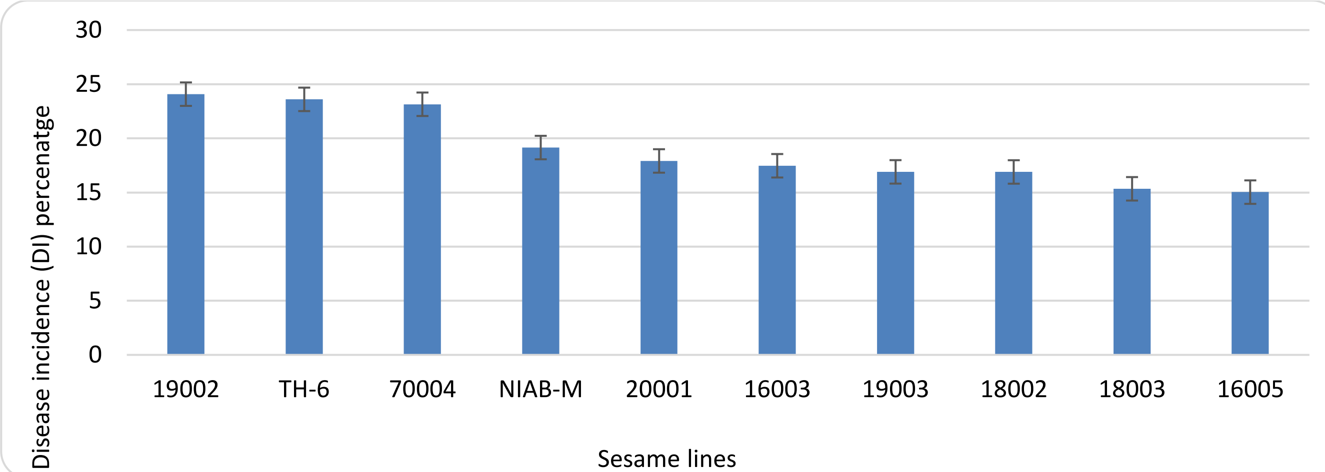

Figure 1. Graph showing mean values of Charcoal rot disease incidence (DI) percentage in sesame lines.

Effect of Fungicides on Growth of *M. phaseolina* Pathogen Under Laboratory Conditions

The colony growth (cm) of fungus (*M. phaseolina*) was measured after one, two, and three days for control and six fungicide treatments (Ridomil Gold, Cabrio Top, Shincar, Captan, Nativo, and Topsin-M) applied in three concentrations of 150, 250, and 350 ppm. At 150 ppm concentration, the mean values were 0.86 cm, 1.23 cm, 1.73 cm, 2.2 cm, 2.53 cm, 2.93 cm, and 6.1 cm for Nativo, Cabrio Top, Topsin M, Ridomil Gold, Captan, Shincar, and Control respectively. The mean colony growth was found as 0.46 cm, 0.6 cm, 0.76 cm, 1.06 cm, 1.3 cm, 1.63 cm, and 6.1 cm for Nativo, Cabrio Top, Topsin M, Ridomil Gold, Captan, Shincar, and Control respectively at concentration of 250 ppm. Similarly, the mean growth values recorded at 350 ppm concentration were as follows: 0.26 cm, 0.4 cm, 0.56 cm, 0.86 cm, 1.1 cm, 1.23 cm, 6.1 cm for Nativo, Cabrio Top, Topsin M, Ridomil Gold, Captan, Shincar, and Control respectively. The mean data is presented in figure 2.

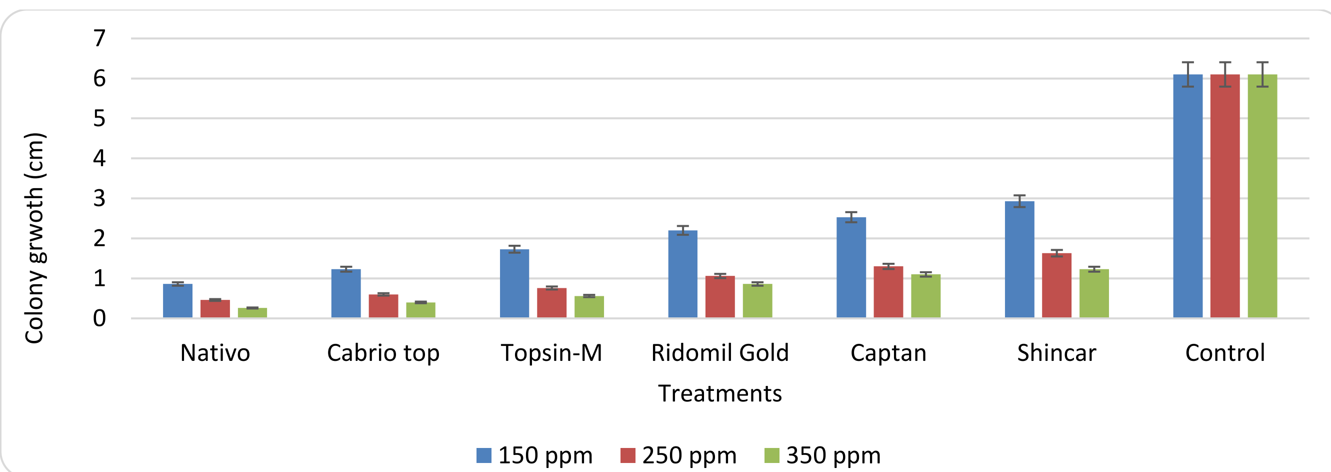

Figure 2. Mean values of colony growth (cm) at normal condition (control) and three concentration levels of fungicides.

After one day of treatment application, the mean growth (cm) of Nativo, Cabrio Top, Topsin M, Ridomil Gold, Captan, Shincar, and Control was measured as 0.23 cm, 0.3 cm, 0.43 cm, 0.63 cm, 0.76 cm, 1.1 cm, 5.66 cm respectively. After two days, the mean values were 0.53 cm, 0.8 cm, 1.1 cm, 1.6 cm, 1.93 cm, 2.23 cm, and 6.16 cm for Nativo, Cabrio Top, Topsin M, Ridomil Gold, Captan, Shincar, and Control respectively. Similarly, the growth was 0.83 cm, 1.13 cm, 1.53 cm, 1.9 cm, 2.23 cm, 2.46 cm, and 6.46 cm Nativo, Cabrio Top, Topsin M, Ridomil Gold, Captan, Shincar, and Control after three days. The results are presented in figure 3.

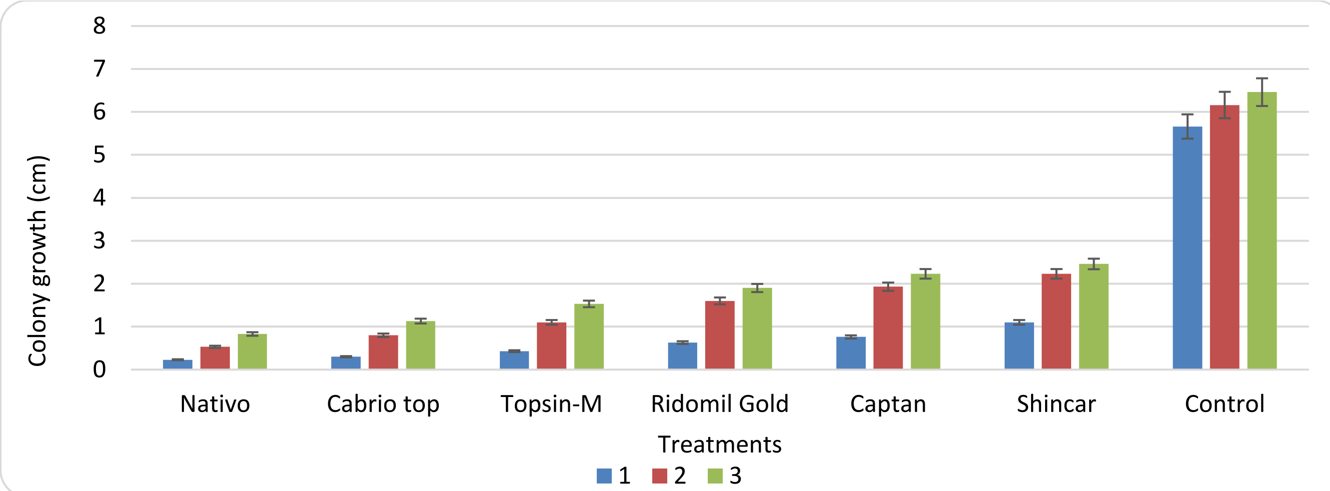

Figure 3. Mean values of colony growth (cm) at normal condition (control) and days after application of fungicides treatments.

After one day of treatment application, the mean growth was observed to be 1.65 cm, 1.2 cm, and 1.05 cm at concentrations of 150 ppm, 250 ppm, and 350 ppm. Whereas, the growth was recorded as 2.62 cm, 1.91 cm, and 1.61 cm after two days at concentrations of 150 ppm, 250 ppm, and 350 ppm respectively. Similarly, the growth was 3.25 cm, 2 cm, and 1.84 cm after three days at concentrations of 150 ppm, 250 ppm, and 350 ppm respectively. The mean values are visualized in the form of graph in figure 4.

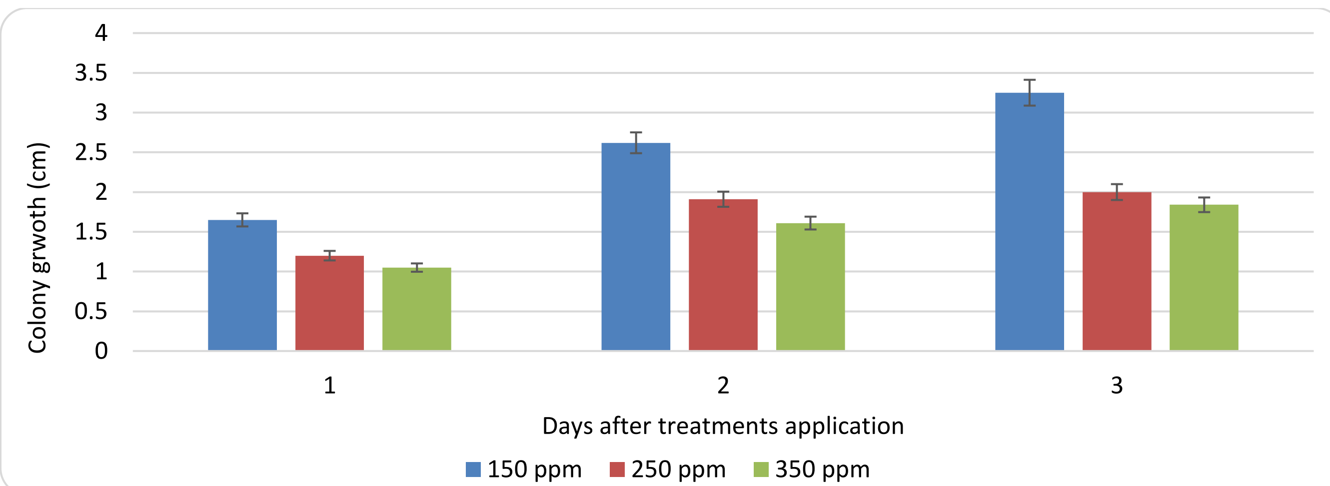

Figure 4. Interaction effect of concentration levels (ppm) and days on mean values of colony growth (cm).

Effect of Fungicides on Charcoal Rot Disease Incidence (%) under Field Conditions

For management of charcoal rot of sesame, the effectiveness of six fungicides, Nativo, Cabrio Top, Topsin M, Ridomil Gold, Captan, and Shincar in the most effective concentration of 350 ppm was also evaluated under field conditions. Each treatment including control with five replications was applied near root zone of sesame plants. A highly susceptible sesame cultivar TH-6 was grown under randomized complete block design (RCBD) with P-P and R-R distance of 45 cm and 75 cm respectively.

As compared to control (77.8%), the mean disease incidence after applying Shincar, Captan, Ridomil Gold, Topsin-M, Cabrio Top, and Nativo was found to be 62.24%, 53.53%, 44.1%, 33.68%, 16.86%, and 11.08% respectively. It can be concluded that Nativo effectively controlled the spread of disease in sesame. Whereas, the fungicide Shincar was found to be least effective in controlling the disease. The mean values are presented in graphical form in figure 5.

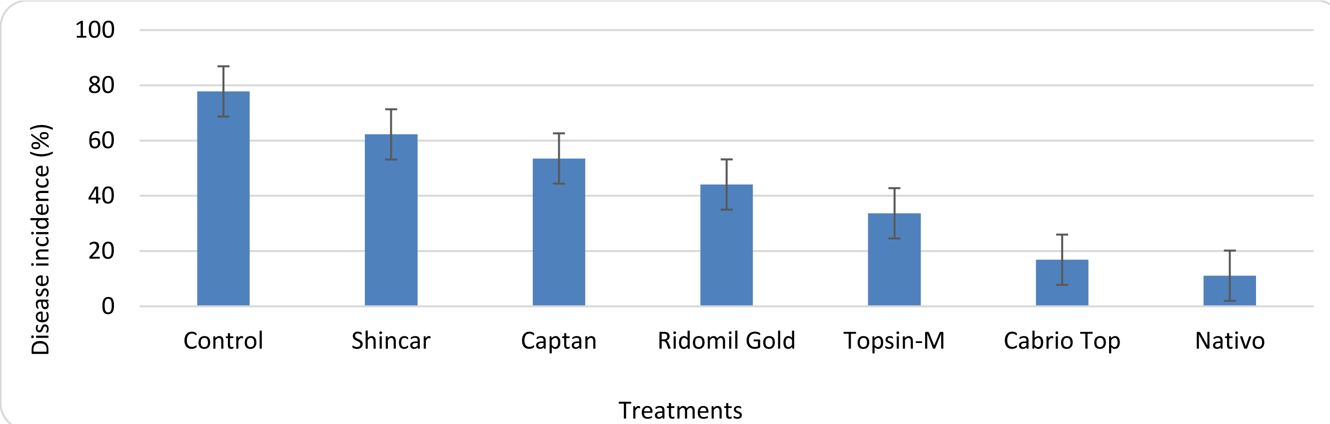

Figure 5. Mean values of disease incidence (%) in normal condition (control) and under fungicidal treatments.

After ten days, the minimum disease incidence was found for Nativo (11.83%) followed by Cabrio Top (20.93%), Topsin M (39.16%), Ridomil Gold (50.46%), Captan (62.26%), and Shincar (68.33%). After twenty days, the disease incidence was recorded as 11.46%, 18.33%, 32.83%, 42.83%, 50%, 62.67%, and 77.33% for Nativo, Cabrio Top, Topsin M, Ridomil Gold, Captan, and Shincar respectively. After thirty days, the disease incidence for Nativo, Cabrio Top, Topsin M, Ridomil Gold, Captan, and Shincar was 9.96%, 11.33%, 29.05%, 39%, 48.33%, and 55.73% respectively (table 4.13). The mean values are presented in a graph shown in figure 6.

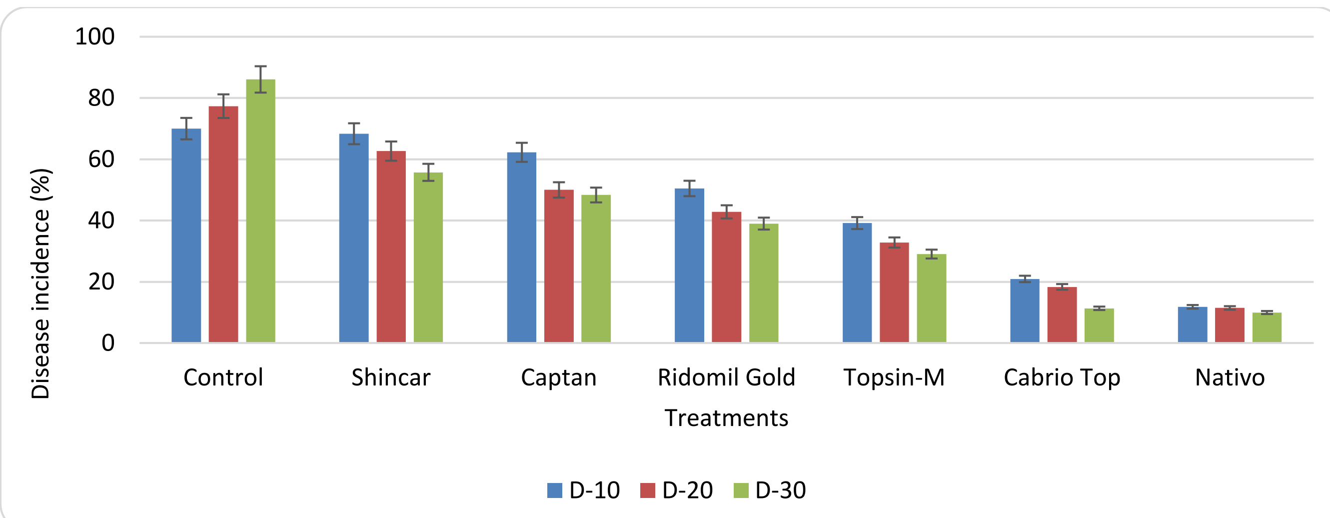

Figure 6. Mean values of disease incidence (%) in relation with treatments and days.

DISCUSSION

Charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goid, poses a significant threat to sesame crops worldwide, resulting in substantial losses. This fungal pathogen becomes more destructive under conditions of elevated temperatures and water stress during the crop growth period (Gupta *et al.*, 2020b). The current research involving two experiments was performed under laboratory and field conditions to study management of charcoal rot of sesame.

In the first experiment, a study evaluated sesame lines for resistance against the charcoal rot pathogen *M. phaseolina*. No sesame line was completely resistant or immune (0-10%) to the charcoal rot disease, but seven lines were moderately resistant (11-20% disease incidence) and three lines were moderately susceptible (21-30%) lines were categorized. Various researchers (Bedawy *et al.*, 2019; Farooq *et al.*, 2019) have consistently reported that complete resistance to this disease with its extensive range of hosts is not currently available.

The second experiment involved the assessment of six fungicidal treatments viz. Shincar, Cabrio Top, Topsin M, Captan, Ridomil Gold, and Nativo in controlling the spread of charcoal rot disease of sesame. The analysis of variance revealed highly significant differences between six fungicide treatments, three concentration levels (150 ppm, 250 ppm, and 350 ppm) and three evaluation timepoints (day-1, day-2, and day-3) in inhibiting the *in-vitro* growth of the fungal phytopathogen *M. phaseolina*. Among the six fungicides tested, Nativo exhibited the highest potential in suppressing the pathogen colony development with a 93% reduction in growth as compared to untreated control. The mean colony diameter with Nativo was merely 0.53 cm as compared to 6.1 cm in control plates. Bashir *et al.* (2017a) examined the *in-vitro* inhibitory effect of Nativo on growing colony of *M. phaseolina* and reported the similar results. The superior inhibitory activity of Nativo can be primarily attributed to the synergistic combination of tebuconazole and trifloxystrobin in its formulation (Kumar *et al.*, 2020).

The two active ingredients with different modes of action allow Nativo to simultaneously inhibit growth and reproduction of *M. phaseolina*. Earlier studies by Kumar *et al.* (2020) reported complete inhibition of *M. phaseolina* mycelial growth by Nativo even at 5 ppm concentration. The colony diameter was merely 0.2 cm with Nativo at 5 ppm as compared to 9 cm in untreated control plates after 7 days of incubation. These results closely align with the current findings and demonstrate the high potency of Nativo even at lower doses of 150-350 ppm in inhibiting *M. phaseolina* growth. The enhanced bio-efficacy of Nativo can be attributed to synergistic interactions between tebuconazole and trifloxystrobin, rather than just additive effects (Amil-Ruiz *et al.*, 2011).

Under field conditions, Nativo provided the highest level of protection against charcoal rot disease in sesame plants with only 11% mean disease incidence compared to 78% in untreated control plants after 30 days. The superior performance aligned closely with the in-vitro growth inhibitory effects of Nativo against M. phaseolina. The potent fungicidal activity can be attributed to efficient uptake and broader systemic distribution of tebuconazole and trifloxystrobin within plant tissues after application. Nativo was applied at a high dose of 350 ppm, which would provide greater amounts of both active ingredients to effectively inhibit various metabolic targets in the pathogen upon uptake (Kumar et al., 2020). Furthermore, the unique combination of local systemic and translaminar mobility of Nativo enables deeper penetration through the leaf surface and efficient translocation to other parts of the plant (Sousa et al., 2023). This allows wider distribution within plant tissues for better management of soil-borne diseases like charcoal rot compared to other fungicides. Nativo reduced disease severity by 63-93% after natural infection across both susceptible and resistant soybean varieties. The current findings in sesame align with the earlier results in soybean and highlight the broad potential of Nativo for controlling charcoal rot caused by M. phaseolina.

STATEMENTS AND DECLARATIONS

Consent for publication. All the authors are consented and approved the submission of the current manuscript.

Competing interests. The authors declare no conflict of interest**.**

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Authors' contributions: MMT, HA and MRB planned, methodology and executed experimental work, MK, SAK and UK writing-draft preparation, SA and MUS review, editing and EUH conducted data analyses.

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