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EXPLOITATION OF NEW CHEMISTRY FUNGICIDES AGAINST CHARCOAL ROT OF SESAME CAUSED BY *MACROPHOMINA PHASEOLINA* IN PAKISTAN

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A B S T R A C T

The current research was conducted to find out the most appropriate concentrations of six fungicides for the management of sesame charcoal rot caused by *Macrophomina phaseolina* under lab and field conditions. The treatments viz. Antracol, Topsin-M, Mancozeb, Score, Topas, Nativo and Control with concentrations of 150, 250 and 350ppm were used with three replications under completely randomized design and randomized complete block design in Lab. and field conditions respectively. The mean colony growth of all treatments expressed that Nativo exhibited minimum colony growth of (0.93 cm) as compared to Score (1.14 cm), Topsin-M (1.42 cm), Mancozeb (1.77 cm), Antracol (2.04 cm), Topass (2.33 cm) correspondingly. The interaction between treatments and concentrations (T×C) showed that used concentrations 150 ppm, 250 ppm and 350 ppm of Nativo abundantly inhibit fungal colony growth upto 1.26 cm, 0.86 cm and 0.66 cm respectively whereas the interaction between treatments and days expressed that after day ninth the minimum colony growth (1.23 cm) was observed for Nativo as compared to all other treatments. Similarly, the interaction between concentrations and days expressed highest fungal colony growth at concentration 150 ppm on day third (2.06 cm), sixth (3.02 cm) and ninth (3.65 cm) but the interaction of treatments, days and concentrations expressed that at 150 ppm concentration, all treatments exhibited minimum colony growth (1.70 to 3.30) cm at third, sixth and ninth day as compared to 250 and 350 ppm concentration respectively with respect to control (6.90 cm). In filed conditions, Nativo exhibited minimum Mean Disease Incidence (12.55%) whereas the interaction between treatments and days showed minimum of 14.95%, 12.82% and 9.90% disease incidence by Nativo as compared to all other treatments including control (66.86%, 77.57% and 87.22%) after day tenth, twenty and thirty. It was concluded that Nativo is significantly inhibiting the colony growth under lab and filed conditions.

Keywords: Sesame charcoal rot, *Macrophomina phaseolina*, invitro and invivo, fungicide evaluation.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is the most significant oilseed crop of Pakistan (Anwar *et al.,* 2013). It is cultivated in tropical and subtropical areas of the world with slightly high temperature (Mensah *et al.,* 2006). The consumption of 100 gram seeds of sesame provide water (1.60ml), food energy (586 kcal), proteins (18.08 g), total lipids (50.87 g), carbohydrate (24.05 g), total dietary fiber (5.5 g), vitamin B_6 (0.816 mg), vitamin A (3 µg), saturated fatty acids (1.252 g), monosaturated fatty acids (3.377g) and polyunsaturated fatty acids (3.919 g) (Nagendra *et al.,* 2012). In the world, it is cultivated on an area of 9398 thousand hectares with total annual production of 4.78 million tons (FAOSTAT, 2015). In

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Pakistan, its area under cultivation is 88 thousand hectares with an average available production of 19.3 thousand tons (Shah *et al.,* 2014).

There are numerous restrains for successful cultivation and production of Sesame crop (Langham and Wiemers, 2002). Among those constraints charcoal rot of sesame caused by *Macrophomina phaseolina* (Tassi.) Goid. is the most imperative disease of this crop (Dinakaran & Mohammed, 2001). The losses caused by this disease under field conditions are 57 percent with 40 percent disease incidence whereas yield losses of 5-100 percent has also observed in Egypt under favorable climatic conditions (EL-Bramawy and Abdul Wahid, 2006). Charcoal rot disease has diminished the production of sesame at about 27 million bushels per year in USA (Chattopadhyay and Sastry, 2002) with an estimated value of $US 146 million (Mame *et al.,* 2014).

Macrophomina phaseolina is necrotrophic and thermophillic phytopathogen in nature (Salik 2007). Although a single specie *phaseolina* is recognized till now within the genus *Macrophomina* but a huge variability in morphology and pathogenicity has seen among various isolates from different hosts (Thippeswamy *et al*., 2003). The pathogen mainly reproduces either through microsclerotia or pycnidia. The fungus spreads promptly through soil as well as seed and enhances the disease severity as soil and air temperature increase from 28-35 °C (Khan, 2007). The characteristic symptoms of this disease are appearance of spindle shaped lesions, light grey center of leaves with dark border, black secondary roots, sudden wilting and death of growing plants (Khalili *et al.,* 2016).

Numerous disease management approaches are available such as resistant varieties, crop rotation, cultural practices, biological control method, soil solarization, systemic induce resistance and minimum supply of soil moisture to diminish the disease incidence of charcoal rot of sesame caused by *Macrophomina phaseolina* but these approaches require highly proficient accuracy in measurements as well as long time is required (Infantino *et al.,* 2006). In the meanwhile, adequate application of systemic fungicides against this disease is an appropriate and easy method to practice, prepare solutions, soil drenching and application with irrigation which is quick in action (El-Fiki *et al.,* 2004). Farmers judiciously apply fungicides to protect plants along with enhanced production, yield and economic return (Arriel *et al*., 2007). The fungicide application possesses relatively low cost and more effectiveness on crops to hinder disease losses (Azeez and Morakinyo , 2010).

Plant protective measures for soil born diseases depend upon an application of systemic fungicides. The effective management approaches are much imperative to diminish the disease incidence caused by soil born thermophillic plant pathogens which is the most pivotal part of the current research. Moreover, environmentally friendly systemic fungicide is the demand of the existing era. Therefore, it is need of the hour to find out an appropriate concentration of systemic fungicides against charcoal rot of sesame. Thus, the most effective concentrations of systemic fungicides were exploited in the current research to diminish disease losses and prevent sesame plants.

MATERIALS AND METHODS

Koch's postulate assessment for *Macrophomina phaeiolina:* Diseased plants with characteristic symptoms of Charcoal rot disease were collected from filed and brought in the oilseeds pathology laboratory for isolation of pathogen. Infected roots were washed thoroughly with tab water and cut into small pieces of 4-6 mm and surface sterilized with 1% NaOCl. The roots were dried by keeping on sterilized filter paper in the petri plate. At least 2-3 pieces of infected roots were placed in petri plate containing Potato Dextrose Agar (PDA) medium. The plates were incubated at ± 25 ^{o}C for 48-72 hours for fungal growth (Sarwar *et al.,* 2005). Then purified colonies of *Macrophomina phaseolina* were identified under stereomicroscope (Soesanto *et al.,* 2011).

Sick field preparation: The most susceptible variety of Sesame i.e. TH-6 was grown in Plant Pathology Research Area of Oilseeds Research Institute, Faisalabad. Three successive drenching of pure culture of *Macrophomina phaseolina* were carried out with irrigation after 15 days interval. Sterilized water @ 3-4 ml was poured on 7-10 days old culture, petri plate was shaked gently and transferred in 250 ml beaker. The no. of spore were counted through haemocytometer by adjusting the spore suspension of 1×10^6 spores/ml of H_2O (Sarwar *et al.,* 2005). After the establishment of disease symptoms, the diseased plants were ploughed in the soil and irrigated to enhance decomposition of plant debris and creating conditions for maximum fungal growth.

Exploitation of antifungal potential of different fungicides against *Macrophomina phaseolina* in Laboratory: Six fungicides viz. Antracol, Topsin-M (thiophanate methyl), Mancozeb, Score (Difenconazol), Topas and Nativao (Trifloxystrobin) were assessed through poisoned food technique at three different concentrations (150, 250 and 350 ppm). These fungicides were mixed in PDA medium by putting an appropriate quantity from stock solution. The amended PDA medium with measured quantity of 20-25 ml was poured in petri plates (9 cm dia.) under laminar flow chamber. Each plate was replicated three times under completely randomized design (CRD) and incubated at 25 ± 2 °C for 10 days. The colony growth was measured after 3, 6 and 9 days. T_1 = Antracol, T_2 = Topsin-M,(Thiophanate methyl 70% w/w), T_3 = Mancozeb, T_4 = Score, (Difenconazol 250 g/ l), T_5 = Topas, T_6 = Nativo, (Tuboconazole 50% w/w + Trifloxystrobin 25% w/w) and T_7 = Control.

Evaluation of Fungicides in Field conditions against Charcoal rot of Sesame: Most effective concentrations of six fungicides Antracol, Topsin-M (thiophanate methyl), Mancozeb, Score (Difenconazol), Topas and Nativao (Trifloxystrobin) with a control treatment were evaluated against Charcoal rot disease of Sesame caused by *Macrophomina phaseolina* under field conditions. Susceptible to moderately susceptible varieties/ advanced lines of sesame were collected from Oilseeds Research Institute, AARI, Faisalabad and sown in sick field under randomized complete block design. The recommended P×P=45 cm and R×R=75 cm distance, fertilizer (60:60:75 NPK) was kept under consideration. Each treatment including control were drenched near root zone with three replications to visualized the impact of fungicides on charcoal rot disease. Data of disease incidence (%) was recorded with ten days interval up to maturity of crop (Table 1).

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

Data analysis: All the statistical tests were performed using SAS/STAT statistical software (SAS Institute, 1990). Means were separated by using Fisher's protected least significant difference (LSD) procedure by taking P = 0.05% probability level (Steel, *et al.,* 1997). Analysis of variance (ANOVA), interaction of different treatments and their combinations were developed by using SAS/STAT software package.

RESULTS

Nativo exhibited minimum colony growth (0.93) as compared to Score (1.14), Topsin-M (1.42), Mancozeb (1.77), Antracol (2.04), Topas (2.33) cm respectively as compared to control (Table 2). Interaction between treatments and concentration ((T×C) showed that Nativo expressed minimum fungal colony growth at 150 ppm (1.26), 250 ppm (0.86) and 350 ppm (0.66) cm, followed by Score (1.63, 1.00, 0.80) cm, Topsin-M (2.13, 1.16, 0.96) cm, Mancozeb (2.60, 1.46, 1.26) cm, Antracol (2.93, 1.70, 1.50) cm, Topas (3.33, 2.03, 1.63) cm respectively (Table 3). The interaction between treatments and days showed that all treatment viz. Nativo (0.63), Score (0.70), Topsin-M (0.83), Mancozeb (1.03), Antracol (1.16), Topas (1.50) expressed minimum colony growth after day three as compared to sixth (0.99, 1.20, 1.50, 2.00, 2.33, 2.63 and 6.56) cm and 9^{th} day (1.23 to 6.86) cm respectively (Table 4). Similarly, the interaction between concentrations and days expressed highest fungal colony growth at concentration 150 ppm on day third (2.06 cm), sixth (3.02 cm) and ninth (3.65 cm) respectively as compared to concentration 250 ppm (1.60, 2.31, 2.40 cm) and 350 ppm (1.45 to 2.24 cm) on all days with respect to control (Table 5).

Similarly, the interaction of treatments, days and concentrations expressed that Nativo exhibited minimum disease incidence (0.80, 1.20, 1.80, 0.60, 0.90, 1.10, 0.50, 0.70, 0.80 cm) at all concentrations *viz.* 150ppm, 250ppm and 350ppm etc. after 10, 20 and 30 days as compared to all other treatments i.e. Antracol, Topsin-M, Mancozeb, Score, Topas, Nativo and control (Table 6).

The mean of all treatments expressed that minimum fungal colony growth was observed at Nativo that was 12.55 percent as compared to other treatments *viz.* Score (16.46 %), Topsin-M (32.33 %), Mancozeb (44.32 %), Antracol (52.45 %), Topas (62.39 %) respectively with respect to control (Table 7). The interaction between treatments and days showed that maximum disease incidence was observed at Topas (68.42%, 63.41%, 55.35%) after ten, twenty and thirty days as compared to other treatments such as Antracol (59.26%, 52.38%, 45.72%), Mancozeb (51.67%, 43.80%, 37.50%), Topsin-M (37.50%, 33.33%, 26.17%), Score (19.50%, 16.75%, 13.14%) and Nativo (14.95%, 12.82%, 9.90%) as compared to control (Table 8).

Table 1. Disease rating scale for charcoal rot of sesame caused by *M. phaseolina*

Disease rating	Description	Response	Symbol
0	0	Immune	I
1	1-20 %	Resistant	R
2	21-40 %	Moderately resistant	MR
3	41-50 %	Moderately susceptible	MS
4	51-70 %	Susceptible	S
5	71-100 %	Highly Susceptible	HS

(Monaim and Ismail, 2010)

Table 2. Impact of various *in-vitro* chemicals on colony growth of *Macrophomina phaseolina* (Tassi.) Gold

Sr #	Treatments	Colony growth (cm)
T_1	Antracol	2.0444
T_2	Topsin-M	1.4222
T_3	Mancozeb	1.7778
T_4	Score	1.1444
T_5	Topass	2.3333
T_6	Nativo	0.9333
T_7	Control	6.5000
	LSD	0.0304

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

Table 3. Impact of various chemicals and concentrations on colony growth of *Macrophomina phaseolina* (Tassi.) Goid

Treatments	Colony growth (cm)		
	Concentrations (ppm)		
	C_{150}	C_{250}	C_{350}
Antracol	2.9333C	1.7000G	1.5000H
Topsin-M	2.1333E	1.1667J	0.9667K
Mancozeb	2.6000D	1.4667H	1.2667I
Score	1.6333G	1.0000K	0.8000L
Topass	3.3333B	2.0333F	1.6333G
Nativo	1.2667I	0.8667L	0.6667M
Control	6.5000A	6.5000A	6.5000A
LSD	0.0811		

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$). C_{150} = 150 ppm, C_{250} = 250 ppm, C_{350} = 350 ppm concentrations

Table 4. Impact of various chemical treatments and days on colony growth of *Macrophomina phaseolina* (Tassi.) Gold

	Colony growth (cm)		
Treatments	$^{*}D_3$	$^{**}D_6$	$^{***}D_9$
Antracol	1.1667I	2.3333F	2.6333E
Topsin-M	0.8333L	1.5000H	1.9333G
Mancozeb	1.0333J	2.0000G	2.3000F
Score	0.7000M	1.2000I	1.5333H
Topass	1.5000H	2.6333E	2.8667D
Nativo	0.6333M	0.9333K	1.2333I
Control	6.0667C	6.5667B	6.8667A
LSD	0.0811		

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$). $^{*}D_3$ = Day third, $^{**}D_6$ = Day Sixth, $^{***}D_9$ = Day Ninth

Table 5. Impact of various chemical treatments and days on colony growth of *Macrophomina phaseolina* (Tassi.) Goid

	Colony growth (cm)		
Concentrations	*D_3	**D_6	***D_9
*C_1	2.0571F	3.0286B	3.6571A
*C_2	1.6000G	2.3143D	2.4000C
*C_3	1.4571H	2.0143F	2.2429E
Control	6.0667C	6.5667B	6.8667A
LSD	0.0461		

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$). *1st = 150ppm, *2nd = 250ppm and *3rd = 350ppm similarly *D_3 = Day third, **D_6 = Day Sixth and ***D_9 = Day Ninth expressing colony growth at consecutive days

Table 6. The impact of chemicals, days and concentrations on the development of charcoal rot of sesame

Treatments	DISEASE INCIDENCE (%)								
	*C_1			*C_2			*C_3		
	D_1	D_2	D_3	D_1	D_2	D_3	D_1	D_2	D_3
Antracol	1.70QR	3.30J	3.80H	1.00XY	2.00NO	2.10N	0.80Za	1.70QR	2.00NO
Topsin-M	1.10WX	2.10N	3.20J	0.80Za	1.30UV	1.40TU	0.60bc	1.10WX	1.20VW
Mancozeb	1.50ST	2.80K	3.50I	0.90YZ	1.70QR	1.80PQ	0.70ab	1.50ST	1.60RS
Score	0.90YZ	1.60RS	2.40LM	0.70ab	1.10WX	1.20VW	0.50c	0.90YZ	1.00XY
Topass	2.30M	3.70H	4.00G	1.30UV	2.30M	2.50L	0.90YZ	1.90OP	2.10N
Nativo	0.80Za	1.20VW	1.80PQ	0.60bc	0.90YZ	1.10WX	0.50c	0.70ab	0.80Za
Control	6.10E	6.50C	6.90A	5.90F	6.90A	6.70B	6.20DE	6.300D	7.00A
LSD	0.1611								

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$). *C_1 = 1st Concentration (150 ppm), *C_2 = 2nd Concentration (250 ppm), *C_3 = 3rd Concentration (350 ppm) whereas D_1 = Fungal colony growth after 10 days, D_2 = Fungal colony growth after 20 days and D_3 = Fungal colony growth after 30 days.

Table 7. Impact of different *in-vivo* fungicides on disease incidence due to charcoal rot of sesame

Sr. No.	Treatments	**Mean DI (%)
T_1	Antracol	52.453C
T_2	Topsin-M	32.333E
T_3	Mancozeb	44.324D
T_4	Score	16.466F
T_5	Topass	62.393B
T_6	Nativo	12.557G
T_7	Control	77.217A
LSD 0.1187		

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$). **Mean DI = Arithmetic mean of Disease incidence in percentage

Table 8. Impact of treatments and days on disease incidence under field conditions

Treatments	Disease incidence (%)		
	Days		
	D_{10}	D_{20}	D_{30}
Antracol	59.260F	52.380H	45.720J
Topsin-M	37.500L	33.330M	26.170N
Mancozeb	51.670I	43.803K	37.500L
Score	19.500O	16.757P	13.140R
Topass	68.420C	63.410E	55.350G
Nativo	14.950Q	12.820S	9.900T
Control	66.860D	77.570B	87.220A
LSD 0.2528			

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$). D_{10} = Disease incidence in percentage after day 10 , D_{20} = Disease incidence in percentage after day 20, D_{30} = Disease incidence in percentage after day 30

DISCUSSION

Charcoal rot of sesame caused by *Macrophomina phaseolina* (Tassi) Goid. is a devastating disease in sesame growing areas of Pakistan (Salik, 2007). The fungus causes huge losses and harm the crop significantly in a wide range of soil temperatures from 25°C to 35°C (Sagir *et al.,* 2009) which causes yield losses of 5-100% under an epidemic conditions. Similarly, disease incidence of 40% was also observed on sesame which consequently reduces the yield upto 57% under sever conditions (EL-Bramawy and Wahid, 2006).

The sclerotia of pathogen survives in the soil, crop residues and on seed which cause characteristic symptoms such as sudden wilting, destruction of fibrous root, blackening of stem and roots (El-Fiki *et al.,* 2004a). In the absence of resistant germplasm against virulent pathogen, the utilization of systemic fungicides is a potential approach to diminish the inoculum density of soil borne diseases (Reznikov *et al.,* 2016).

In the current research six fungicides *viz.* Nativo, Score, Topsin-M, Mancozeb, Antracol and Topas were assessed against Charcoal rot disease of sesame caused by *Macrophomina phaseolina* (Tassi) Goid. with different concentrations. Nativo expressed minimum fungal colony growth (1.26 cm at 150 ppm concentration by disrupting the metabolism as well as by hampering the growth and development of pathogen. It binds through covalent bond formation with sclerotia of pathogen and interrupts its ionic concentration (El-Fiki *et al.,* 2004b). The findings of the current research are in line with the studies of Kumar *et al.* (2016) who assessed Trifloxystrobin 25% + Tebuconazole 50% @ 5, 10, 15, and 25 ppm against *Macrophomina phaseolina* and observed that Nativo expressed significant reduction in colony growth as compared to other fungicides. Chennakesavulu *et al.* (2013) evaluated six fungicides i.e. carbendazim, tebuconazole, propiconazole, hexaconazole, mancozeb and cheshunt with five concentrations of 50, 100, 250, 500 and 1000 ppm against charcoal rot disease pathogen namely *Macrophomina phaseolina* through poison food technique and diminished that carbendazim, Tebuconazole and propiconazole completely inhibited the mycelial growth of the pathogen even at 50 ppm as compared to all other concentrations.

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