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COMPARATIVE EFFICACY OF FUNGICIDES AND SALICYLIC ACID AGAINST *MACROPHOMINA PHASEOLINA* CAUSING CHARCOAL ROT UNDER LABORATORY CONDITIONS

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A B S T R A C T

To date, in Pakistan, no fungicide has been registered against *Macrophomina phaseolina*. This study was designed to check the efficacy of two fungicides and salicylic acid against *M. phaseolina* under laboratory conditions. Both fungicides and salicylic acid had given significant results inhibiting the mycelial growth compared to control. The application of Nativo @ 6 mM resulted maximum inhibition in mycelial growth at 3rd day (69.32%), at 5th day (70.84) and at 7th day (73.82%) than control. Fungal growth reduced with the increase in the concentration of fungicides and salicylic acid and decreased with decrease in concentration. The application of Success @ 6mM gave a good result but lower than the Nativo. Both the fungicides and Salicylic acid gave maximum inhibition of fungal growth at the concentration of 6mM. Mycelial growth recorded at 7th day with the treatment of salicylic acid @ 6mM was 55.04% as compared to the control followed by Success 70.23%. The results showed Nativo @ 6 mM may be the best for the control of charcoal rot under laboratory conditions.

Keywords: Sunflower, Charcoal rot, *Macrophomina phaseolina.*

INTRODUCTION

Charcoal rot which causes *Macrophomina phaseolina* affects different crops in Pakistan among which sunflower is the most affected crop (Ghaffar, 1988). Sunflower scientifically named as *Helianthus annuus* L. belongs to the family *Asteraceae* and genus *Helianthus.* Total production of sunflower in Pakistan during 2010 was 325,478 tons which has been decreased to a larger extent in 2017 during which production recorded was 104,000 tons (FAOSTAT, 2017). In Pakistan charcoal rot of sunflower was first time reported in 1984 from Faisalabad (Mirza, 1984). The first survey of sunflower crop was conducted by Mirza and Beg, (1983) in the northern and central areas of Pakistan and according to them up to 90% losses of yield were caused by *Macrophomina phaseolina* (Ullah *et al*., 2011). This pathogen caused discoloration of seed and increased free fatty acids (Ataga and Akueshi, 1986).

This fungus belongs to the genus Macrophomina and family Botryosphaeriaceae (Crous *et al*., 2006). Viability of this fungus as sclerotia may extend up to 4 years in crop residues and soil. Charcoal rot disease caused by this fungus is followed by dry weather, wet weather in spring that is followed by hot in reproductive growth stages (Markell *et al*., 2015). This pathogen survives in a wide range of soil temperature; from 25oC to 35oC (Sagir *et al*., 2009) and causes huge yield losses and destroy the crop significantly; under epidemic conditions it tend to cause 5-100% yield losses

M. phaseolina is pathogen of different crops like chickpea, sesame, cowpea, clusterbean, peanut, maize, soybean, sorghum, common bean, mungbean, cotton and sunflower (Dhingra and Sinclair 1977; Lodha *et al*., 1986; Diourte *et al*., 1995). Softwood and forest trees like *Pseudotsuga, Abies, Pinus, Cassia*, weed species and medicinal plants and fruit trees (*Cocos nucifera*, *Coffea*

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spp., *Citrus* spp., *Leucaena* spp., *Ziziphus mauritiana*) are found to be the other hosts of this fungus. It also attacks on soybean in South and North America, Asia, Australia, Africa and European continents (Gupta *et al*., 2012).

Hyphae of *M. phaseolina* first invade the plant's cortical tissues that results in the formation of sclerotia and eventually causing stem rot disease. Sclerotia and mycelia produced are gray-black in color, and the symptoms can be seen on infected area. The conidia of *M. phaseolina* are aseptate, thin-walled, elliptical and hyaline. When the conditions are favorable hyphae of the fungus grow and produce the sclerotia that infect the roots of host crop through chemical softening or mechanical pressure by penetrating into plant cell wall. The initial symptoms of disease show leaf yellowing that results in wilting and eventually cause plant death (Islam *et al*., 2012).

Till date, different disease management strategies, viz. physical, cultural, regulatory, chemical (fungicides) and biological (Ullah *et al*., 2011) have been implemented to combat and eradicate the phytopathogenic fungi (Khalili *et al*., 2016). However, these methods are not yielding desirable results. *Chinopodium* extracts of three different species *C. album* L., *C. murale* L. and *C. ambrosioides* L. were used to found helpful against growth of *M. phaseolina* that causes charcoal rot in sunflower and for this purpose leaf, stem, root and inflorescence were used (Javaid and Amin, 2009). All above mentioned methods are applied as precautionary measures and seem to be effective only when these are employed well prior to disease (Kata, 2000). Once a pathogen has arrived and disease has appeared; these strategies become impractical or seem to be ineffective. In that situation, chemical control offers a good choice to growers to control the disease. Chemical fungicides and many pesticides are being used since long period of time and providing effective, economic and quick management of this disease (Ullah *et al*., 2011). Thus, this study was conducted to find out the most effective chemical and their appropriate concentration against *M. phaseolina*.

MATERIALS AND METHODS

This study was performed to check the efficacy of Salicylic acid and two fungicides (Nativo and Success) against pathogen of charcoal rot. The experiment was performed at the Plant Pathology lab. College of Agriculture, University of Sargodha.

Collection of diseased samples isolation and purification: The sunflower samples infected with charcoal rot were collected from the Research Area of College of Agriculture University of Sargodha based on visual symptoms. They were kept in sterilized zipper bags. The infected parts were thoroughly cleaned and washed with 1% sodium hypochlorite solution for 1-2 minutes. The samples were later on placed on potato dextrose agar (PDA). The plates were incubated in darkness at 28±1˚C. The colonies of *M*. *phaseolina* which appeared after 24-48 h were purified by hyphal-tip isolation technique (Aboshosha *et al*., 2007) and a single colony was selected for morphological determination using the identification key (Chang *et al*., 2006) and preserved on PDA slants at 4˚C for future use.

Pathogenicity: The pathogenicity of *M. phaseolina* was confirmed by artificial inoculation of this pathogen on healthy seeds in pot experiment following Koch's postulates using seed inoculation technique recommended by Kataria and Grover, (1976). For this purpose, healthy sunflower seeds were coated with 7 days old culture of fungus on PDA filled petri plates. These inoculated seeds were sown in cemented pots, and un-inoculated seeds served as control treatment. Pots were watered regularly as required. Symptoms on the seedlings were observed and compared with the collected samples.

***M. phaseolina* multiplication for pot assay:** Seeds of sunflower were soaked in water overnight under room temperature i.e 25 ± 1°C. After that, they were air dried and kept in conical flask. Flask mouth was closed by using cotton wool, then aluminium foil was used to wrap the flask and autoclaved for 20 minutes at 121°C and 15 psi. When the temperature lowered down and the flask got cooled, seeds were inoculated with 4 mm mycelial plugs from a 7-days old culture of *M. phaseolina* and incubated for 15 days at 25 ± 1°C. For obtaining uniform colonization, the flask was shaken repeatedly (Iqbal and Mukhtar, 2020). The produced inoculum was used in the pot experiment.

Inoculum preparation: The inoculum for *M. phaseolina* was prepared by culturing the pathogen on PDA media petri- plates. The 10 ml autoclaved distilled water was applied in the petriplates having seven days old culture of *M. phaseolina*. Then the plates were shaken gently, and the obtained suspension was filtered with cheese cloth to remove the fragments of mycelia.

The experiment was performed to check the efficacy of SA and two chemical fungicides, i.e., Nativo and Success against *M. phaseolina* in the Plant Pathology lab. College of Agriculture, University of Sargodha.

Table 1. The chemical composition of Nativo and Success

Sr.#	Nativo	Sr.#	Success
1	Tubeconazole ($C_{16}H_{22}ClN_3O$) mol. Weight (307.8 g/mol)	1	Chlorothalonil ($C_8Cl_4N_2$) mol. Weight (265.91 g/mol)
2	Trifloxystrobin ($C_{20}H_{19}F_3N_2O_4$) mol. Weight (408 g/mol)	2	Metalexyl ($C_{15}H_{21}NO_4$) mol. Weight (279.33 g/mol)

Experiment layout and treatments: The experiment was laid in completely randomized design using the food poison method (Dhingra and Sinclair, 1977). The treatment for Salicylic acid, Nativo and Success were evaluated at different concentrations viz. 2, 4 and 6mM. Three replications of each concentration were used under randomized complete block. The test chemicals were added to the liquefied PDA media. After the solidification of medium, each treatment was applied on three plates separately with the inoculation of fungal plug of actively growing *M. phaseolina*. Plates were placed in the incubator @ 25˚C ± 2 and the mycelial growth was recorded after 3, 5 and 7 days respectively till the control plates were fully covered with the fungus.

Mycelial growth: Data for mycelial growth was recorded after 3rd, 5th and 7th days from each treatment and plate separately, on visual observations then the average data was computed for each treatment. Each petri plate was divided into four equal parts by marking two intersecting lines on the back of plate with permanent marker for the sake of data recording of growing mycelium. The percent inhibition of fungal growth by fungicides was calculated according to (Sireesha and Venkateswarlu, 2013).

$$\text{Percentage inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

STATISTICAL ANALYSIS

Statistical data analysis was performed using Statistix 8.1. Analysis of variance was performed to note down the effect of treatments. Treatment means were compared by Least Significant Difference (LSD) test (Steel *et al*., 1997) under completely randomized design.

RESULTS

Mycelial growth inhibition at 3rd day after treatment: The efficacy of the applied chemical can be estimated by the *M. phaseolina* mycelial growth. More the mycelial growth less will be the efficacy of the applied chemicals. The minimum mycelial growth (13.62 mm) with maximum mycelial growth inhibition (69.31 %) was observed from the application of Nativo @ 6mM followed by Success @ 6mM (14.43 mm with 67.50%, mycelial growth inhibition) compared to control. The maximum mycelial growth (43.25 mm) with minimum inhibition of mycelial growth (2.59 %) was recorded from the application of Salicylic acid @ 2mM compared to control. The mean comparison showed insignificant differences among treatment means (Figure1).

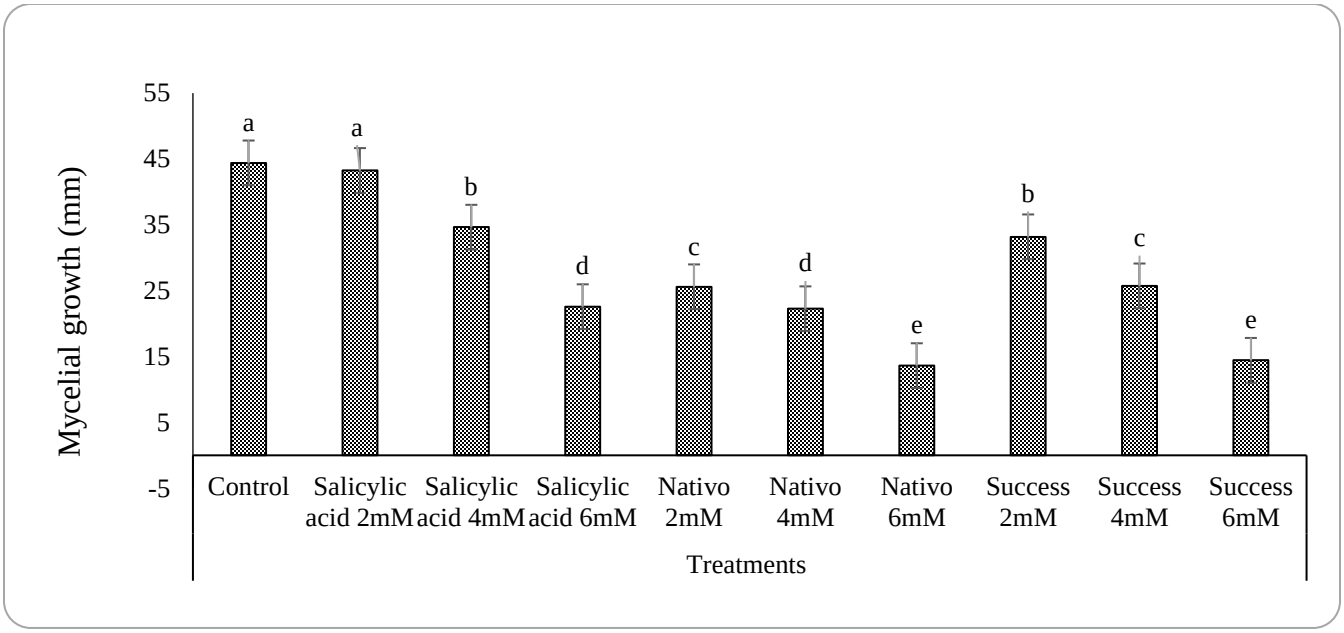

Figure 1. Mean performance of different chemicals against *M. phaseolina* mycelial growth on 3rd day after the treatment

Mycelial growth inhibition at 5th day after treatment: The minimum mycelial growth (15.73 mm) with maximum mycelial growth inhibition (70.84%) was seen from the application of Nativo @ 6mM followed by Success @ 6mM (18.84 mm with 65.07% of mycelial growth inhibition) compared to control. The maximum mycelial growth (49.26 mm) with minimum inhibition of mycelial growth (21.29%) was recorded from the application of Salicylic acid @ 2mM compared to control). The mean comparison showed significant differences among treatment means except for Salicylic acid @ 6mM and Nativo @ 4mm which showed insignificant differences for the mycelial growth (Figure 2).

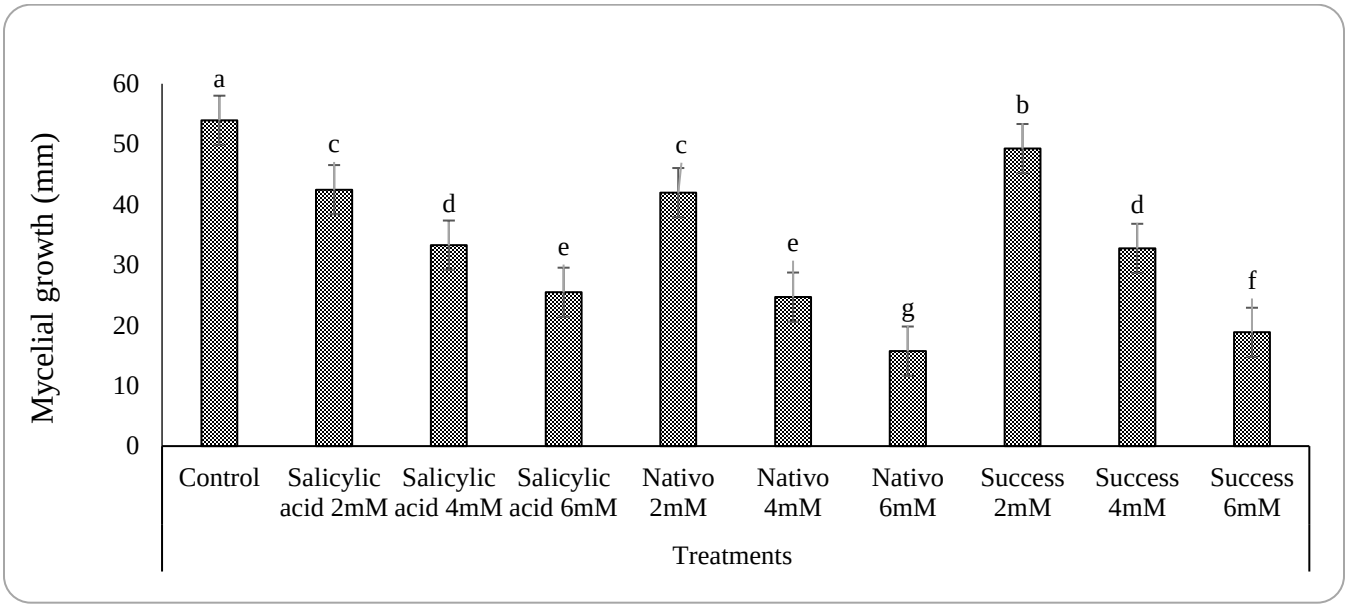

Figure 2. Mean performance of different chemicals against *M. phaseolina* mycelial growth on 5th day after the treatment

Mycelial growth inhibition at 7th day after treatment: The minimum mycelial growth (16.60 mm) with maximum mycelial growth inhibition (73.82%) was recorded from the application of Nativo @ 6mM followed by Success @ 6mM (18.88 mm with 70.23% of mycelial growth inhibition) compared to control. The maximum mycelial growth (54.30 mm) with minimum inhibition of mycelial growth (14.38%) was recorded from the application of Salicylic acid @ 2mM compared to control. The mean comparison showed significant differences among the treatment means (Figure 3).

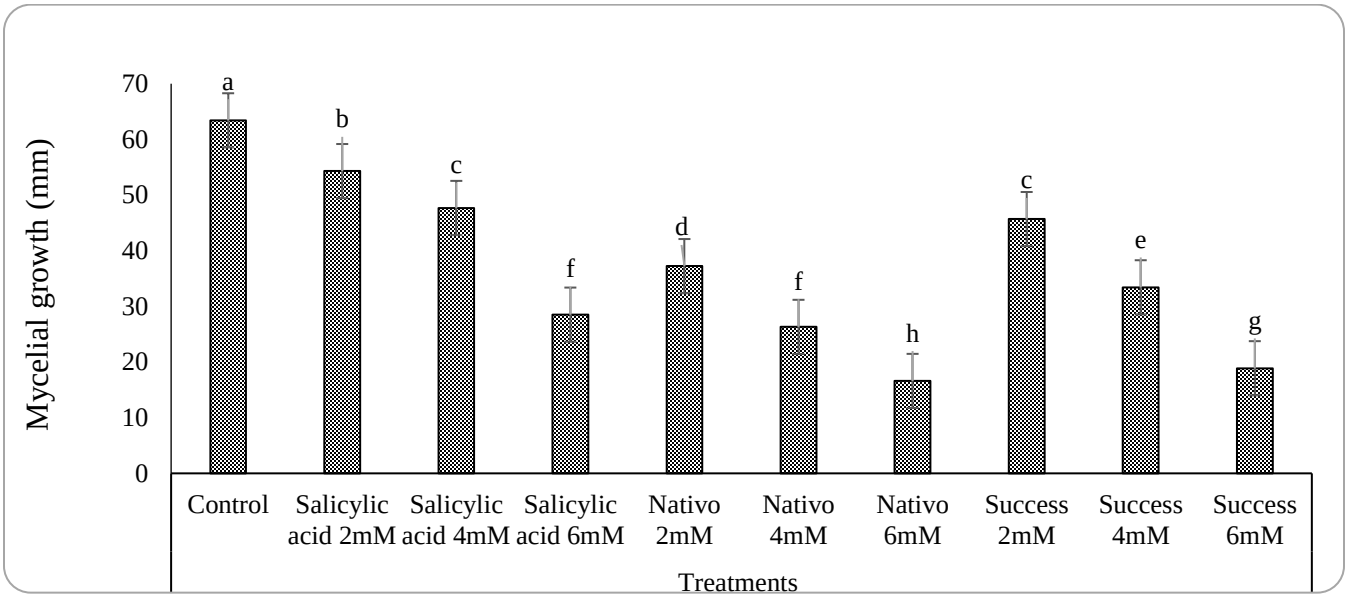

Figure 3. Mean performance of different chemicals against *M. phaseolina* mycelial growth on 7th day after the treatment

DISCUSSION

Our results showed that Nativo expressed least mycelial growth and highest inhibition percentage @ 6mM; 73.82 % at 7th day as compared to the control. The results of this study are in line with the research of Bashir *et al*., (2017) who tested six different fungicides (Mancozeb, Topas, Topsin-M, Antracol, Score and Nativo @ 150, 250 and 350ppm) for their effectiveness against *M. phaseolina* under laboratory conditions and observed that Nativo showed significant results with reduction in the colony growth to a larger extent as compared to other fungicides tested. The results reported by Kumar *et al*., (2016) are also supportive to present study, the authors tested four concentrations (5, 10, 15 and 25 ppm) of Tebuconazole 50% + Trifloxystrobin 25% for the management of charcoal rot pathogen (*M. phaseolina)*. Their results showed that Nativo exhibited great potential against *M. phaseolina* in comparison with the other treatments. Chennakesavulu *et al*., (2013) who assessed Tubeconazole, Mancozeb, Carbendazim, Hexaconazole and Propiconazole @ of 50, 100, 250, 500 and 1000ppm using food poison technique against *M. phaseolina* and concluded that three fungicides i.e. Propiconazole, Carbendazim and Tubeconazole @ 50ppm completely retarded the fungal growth in comparison with the other concentrations used in the research. Parmar *et al*., (2017) worked for the management of fungal pathogen *M. phaseolina* and assessed that the maximum mycelial growth inhibition (99.97%) was showed by carbenzadine at 550 and 250 ppm concentrations. Swamy *et al*., (2018) used fungicides; tricyclazole 18 % + mancozeb 62 %, zineb 68 % + hexaconazole14 % and carbendazim 25 % + mancozeb 50 %, mancozeb 63 % + carbendazim 12 % and recorded highest mycelial growth inhibition (100%).

CONCLUSIONS

The present studies concluded that among all the tested fungicides, Nativo @ 6 mM gave maximum control of charcoal rot under laboratory conditions. This can be augmented in the successful integrated management of charcoal rot of sun flower.

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Habiba U. Rehman	:	Plan and executed the experiment as well as recorded data
Muhammad U. Ghazanfar	:	Conceive the idea, facilitated, guided and supervised the experiment
Muhammad S. Goraya	:	Finalized the manuscript