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# In vitro management of citrus melanose caused by *Phomopsis citri* through commercially available fungicides

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## Abstract

Citrus melanose, caused by *Phomopsis citri* is a serious disease that can affect trees at any age. Symptoms include small, dark brown to black colored spots on the fruit, leaves and twigs. Though it does not affect internal fruit quality but market value of the citrus is reduced to a great extent. Samples were collected from an orchard of District Toba Tek Singh, Punjab during March-April 2018 and *P. citri* (PPS-114) was isolated and maintained. Five fungicides (i.e., Amistar Top 325 SC, Emesto Silver 240 FS %, Kocide® 3000, Nativo 75% WG and Score 250 EC) were tested using poison food technique each with 100, 200 and 300 PPM concentrations. Data was recorded on the basis of colony diameter and percent inhibition of mycelial growth. Results indicated that all the tested fungicides had significant antifungal potential against the test fungus and their inhibition is concentration dependent. Among the tested fungicides, Kocide exhibited 100 percent growth inhibition at the highest tested concentration i.e., 300 ppm followed by Nativo which was accounted for 94 per cent reduction of mycelial growth over control. Score and Emesto silver showed 84 and 82 percent growth inhibition respectively. The least growth inhibition over control was observed with Amistar top i,e., 74 percent. It was concluded that Kocide® 3000 is best for in vitro management of *P. citri*.

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## Introduction

In Pakistan, citrus fruit is being produced throughout the country but its maximum production (approx. 98%) is concentrated in the Punjab province. This plant is attacked by a number of pests and diseases during pre & post- harvest stages that resulted in 20-40% loss of the produce. Many fungal, bacterial and viral pathogens cause important diseases in citrus fruit and their effects could be aggravated by unfavorable weather conditions, delayed harvesting, poor marketing and cold storage facilities.

Citrus melanose, caused by *Phomopsis citri* H. Fawc Non (Sacc.) Traverso and Spessa (Teleomorph *Diaporthe citri* F.A. Wolf), is a serious disease that can affect trees at any age (Gopal *et al.*, 2014). Symptoms include small, dark brown to black colored spots on the fruit, leaves and twigs. Damage is superficial and does not affect internal fruit quality. The incidence of melanose usually increases as trees age.

*Phomopsis citri* usually known as a weak pathogen and undergoes saprophytic phase to complete its lifecycle. But its control is not easy and involves several applications of fungicide under field conditions. Data availability on the fungicides mode of action against citrus melanose is very limited (Bushong and Timmer, 2000). Albrigo *et al.*, (2005) recommended protectant copper fungicides for control of *P. citri.* This protectant mode of action of copper fungicides against melanose was also endorsed by Whiteside (1977). Many protectant fungicides, such as copper fungicides chlorothalonil, captan and dithiocarbamates have been registered in Florida USA for many years. However studies revealed that it is not worthwhile to spray the vegetative plant parts including leaves as rapid expansion of tissues reduce the effectiveness of protectant fungicides. Many studies demonstrated local systemic activity of strobilurin fungicides in fruit cuticle and leaf lamina against foliar fungal diseases (Baldwin, 1996; Ypema and Gold, 1999). Some sterol-biosynthesis-inhibiting (SBI) fungicides inhibit the sporulation that results in reduced subsequent disease (O'Leary and Sutton, 1986; Schwabe, *et al.*, 1984; Wilcox, 1990). Work on post infection mechanism of fungicides revealed that Benomyl efficiently manage the pycnidiospores production on dead twigs rather than having less protectant activity (Whiteside, 1977; Bushong and Timmer, 2000).

No authentic data is available on melanose infection development and management of *P. citri* in Pakistan. So this study is a part of investigations on prevalence, incidence & severity of melanose in different districts of Punjab, Pakistan and management of *P. citri.* This will ultimately help in designing integrated disease management protocol against citrus melanose.

## Materials and methods

*Isolation and Maintenance of fungal culture*

Infected leaves showing the characteristic melanose symptoms were collected from an orchard of District Toba Tek Singh, Punjab during March-April 2018 and transported to Seed Pathology Laboratory, Plant Pathology Section, PPRI, AARI, and Faisalabad. Samples were surface sterilized with 1% NaOCl solution for 5 minutes, thoroughly washed and transferred to 90mm PDA Petri plates under aseptic conditions. Plates were incubated for 5-7 days at 25±2 °C. Single hyphal tip method was used to obtain pure culture of *Phomopsis citri.* Kochs postulates were observed for the confirmation of association of pathogen with the citrus melanose. Cultures were maintained 4 °C for further studies by periodical sub-culturing in PDA slants.

*In vitro activity of fungicides*

To evaluate the *in vitro* effect of different commercial fungicides against the *P. citri,* poison food technique was used. Five fungicides [Amistar Top 325 SC (Azoxystrobin + Difenoconazole), Emesto Silver 240 FS % (Penflufen), Kocide® 3000 (Copper Hydroxide), Nativo 75% WG (Tebuconazole + Trifloxystrobin) and Score 250 EC (Difenoconazole)] were tested each with three concentrations i.e. 100, 200 and 300 PPM respectively. Pre-autoclaved medium was amended with the tested concentrations before pouring into 90 mm Petri plates. No fungicide

was added in control treatment. A plug of 5 mm was used for inoculation from 7 day old actively growing *P. citri* culture. Plates were incubated for 10 days at 25±2 °C. To conduct the experiment, completely randomized design (CRD) was used with five replications of each treatment. Data was recorded on the basis of colony diameter and inhibition of mycelial growth (%) was calculated using the following formula (Gupta and Tripathi, 2011). Data was subjected to analysis of variance (ANOVA) followed by Tukey's HSD (honest significant difference) using SPSS v 15.0 for windows.

$$\text{Percent growth inhibition} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growthincontrol}} \times 100$$

**Results and discussion**

*Phomopsis citri* (isolate PPS-114) associated with citrus melanose was isolated and maintained at 4 °C in the seed pathology laboratory, Plant Pathology Research Institute, Faisalabad. In vitro efficacy of different fungicides to inhibit the growth of *P. citri* was determined by poisoned food technique and the results are summarized in Fig 1.

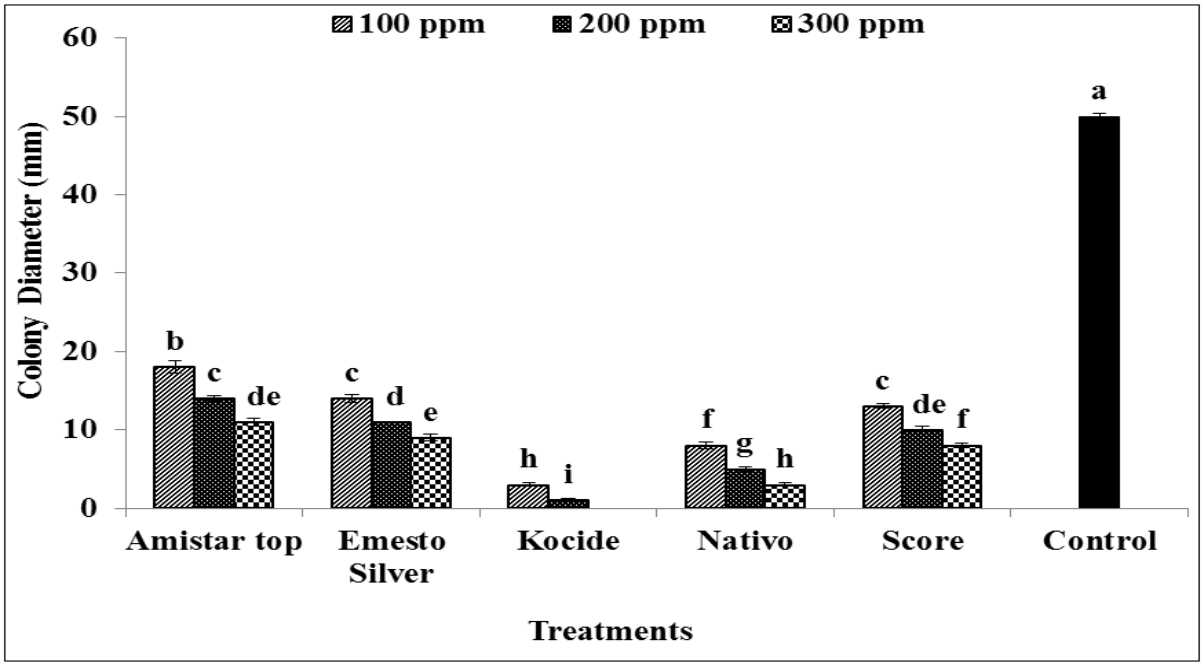


**Fig. 1.** Effect of different fungicides on colony growth of *P. citri*. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD method.

Fungicides were selected on the basis of their effectiveness in previous literature and availability in the market. Results indicated that all the tested fungicides had antifungal potential against the test fungus as the average diameter of colonies of test fungus in was significantly reduced in the poison food plates than that of colony diameter in control plates. It was also noticed that inhibition was concentration dependent and with increase in the concentration of fungicides, mycelial growth of *P. citri* decreased. Among the tested fungicides, Kocide® 3000 exhibited 100 percent growth inhibition at the highest tested concentration i.e., 300 ppm whereas the growth at 200 ppm concentration of kocide is significantly lower i.e., 1.0 mm than other tested fungicides (Fig 2). This was followed by Kocide (100 ppm) and Nativo (300 ppm) recording 3.0 mm of the mycelial growth which was accounted for 94 per cent reduction of mycelial growth over control. Score and Emesto silver at their highest tested concentration i.e., 300 ppm showed 84 and 82 percent growth inhibition respectively. The least growth inhibition over control was observed with Amistar top i,e., 74 percent among the tested fungicides (Fig 2).

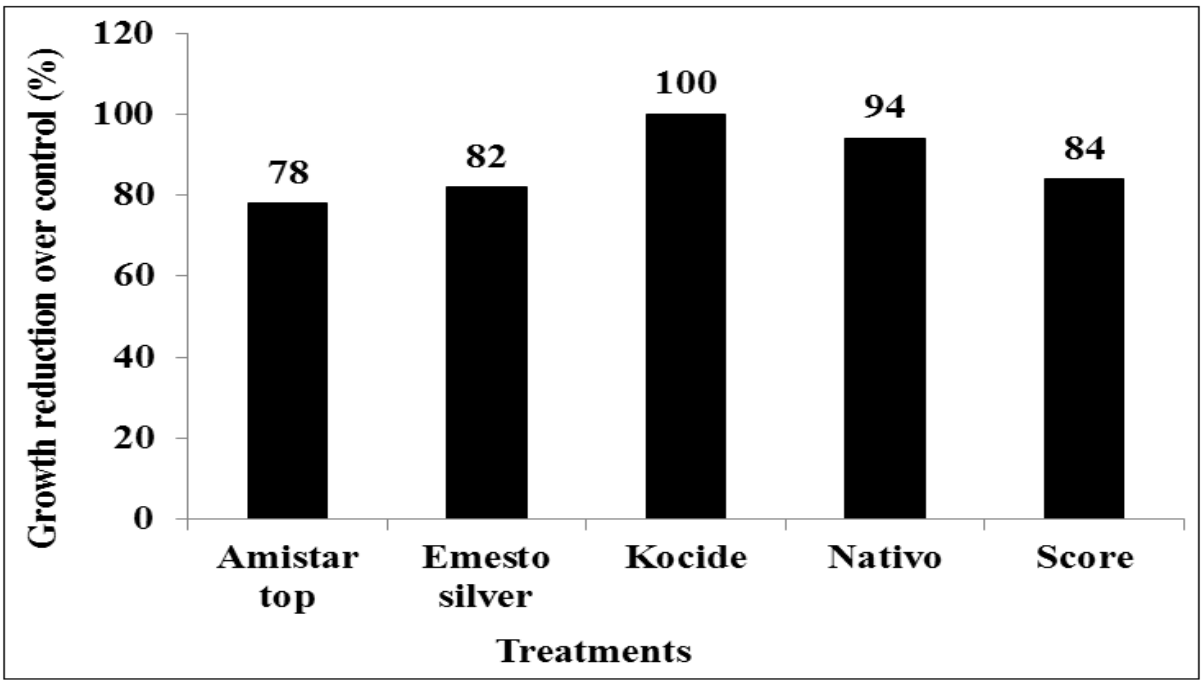


**Fig. 2.** Mycelia growth reduction over control (%) of fungicides against *P. citri*. Vertical bars show means of five replicates.

These results are in line with Mondal *et al.*, (2007) trials conducted for pre and post-infection effectiveness of copper fungicide against melanose. Yang *et al.*, (2011) studied mode of action of different fungicides and reported that copper-based fungicides may disrupt the cellular proteins and have multisite activity. Protectant copper sprays are the only product registered for melanose control but effective for only short periods when applied to rapidly growing foliar (Bushong and Timmer, 2000).

In vitro effectiveness of Difenoconazole, Tebuconazole, Azoxystrobin and Trifloxystrobin in checking the growth of mycelium of *P. citri* on PDA has been reported (Gopal *et al.*, 2014). Triazole and strobilurin fungicides are systemic and have broad spectrum protectant as well as curative effect against plant pathogens. Strong protective and curative activity has been observed by systemic fungicides.

However Zitko and Timmer **(**1997) reported that azoles and strobilurins fungicides are not as effective in controlling melanose. It was revealed in present studies that the strobilurin fungicides, Amistar top is also effective for melanose control but copper fungicides are more cost-effective. Major drawback of copper fungicides is fruit damage when applied in hot weather while strobilurins at that time will avoid this loss and control melanose. Dewdney and Timmer (2011) reported that strobilurins have lower residual toxicity than copper fungicides. Present studies revealed least effective concentration of Kocide and Nativo fungicides under in vitro conditions against *P. citri*. However, this needed detailed investigations of preventive and/or curative activity of selected fungicides under in vivo conditions field because of the involvement of many other factors which may affect the results. These studies are in progress and results will help to achieve the objective of designing the integrated disease management practices against citrus melanose according to the climatic conditions of Punjab, Pakistan.

**Conclusion**

Citrus melanose, caused by *Phomopsis citri* becomes a serious disease that can affect trees at any age and drops the market value of the fruit to a great extent. In vitro efficacy of five fungicides was tested using poison food technique. Among the tested fungicides, Kocide exhibited 100 percent growth inhibition at 300 ppm concentration followed by Nativo which was accounted for 94 per cent. Present studies concluded

that Kocide® 3000 and Nativo performed well under in vitro management of *P. citri* and can be further evaluated for their antifungal activity under in vivo conditions.

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