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Protectant and curative efficacy of different fungicides against citrus melanose caused by *Phomopsis citri* under in vivo conditions

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Abstract

Melanose caused by *Phomopsis citri* pose a major threat to citrus plants that can affect trees at any age and damages fruit, leaves, twigs, branches. If favorable conditions prevail, this disease may result in heavy economic losses. Present study was conducted for *In-vivo* Protectant and curative efficacy evaluation of fungicides against *P. citri*. Five treatments consisting of chemicals viz., Emesto Silver 240 FS % (Penflufen), Kocide® 3000 (Copper Hydroxide), Nativo 75% WG (Tebuconazole + Trifloxystrobin) and Score 250 EC (Difenoconazole) were prepared at recommended doses with three replications and control plants were untreated. Data was recorded on disease incidence (%) and disease reduction over control (%) at weekly interval. In general, all the tested fungicides exhibited significant antifungal potential when applied as protectant while least effective or show no activity as curative. The least disease incidence was observed with Kocide i.e., 12% followed by Nativo (18%) whereas the highest incidence (21%) was recorded by Score and Emesto silver. Percent disease reduction over control was calculated and revealed that Kocide, Nativo, Score and Emesto silver significantly lowers ($P < 0.05$) the incidence of melanose by 80, 71, 65 and 65% respectively. It was concluded that Kocide is best for the management of citrus melanose when applied as protectant under in vivo conditions.

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Introduction

Citrus is a juicy fruit cultivated almost all over the world under diverse climatic conditions. Brazil is the top producer of citrus fruits with an annual production of about 20 million tons followed by China and United States i.e., 19.6 and 10 million tons respectively. Pakistan is the 12th largest producer of citrus and the largest producer of Kinnow in the world. Currently, in Pakistan citrus fruits are grown on an area of 206,569 hectares with production of 2.36 million tons in 2015-16. Citrus fruits are a good source of fiber and antioxidants. Citrus fruits and citric products like juices are a rich source of immune-boosting vitamin C. Vitamin C is good for the elasticity of skin and reducing the severity of cold. Citrus fruits are rich in other minerals like calcium which is necessary for strong bones and potassium which is good for the functioning of the nervous system.

There are several production constraints but pests and diseases cause severe yield losses. Among diseases melanose caused by *Phomopsis citri* (Teleomorph *Diaporthe citri*) has emerged as a major threat during the past few years (Gopal *et al.,* 2014). It can affect trees at any age and damages fruit, leaves, twigs, branches, and in some circumstances, the main trunk of the tree. Damage is superficial and does not affect internal fruit quality (Mondal *et al.,* 2006). On the fruit, leaves and small twigs, small, dark brown to black spots are produced which are raised and rough to touch. The spots are superficial and can be removed with your fingernail. Melanose, are produced only on dead twigs. After dispersal by rain water, infection occurs within 8 to 10 h at an optimum temperature of 25°C (Kramoto and Yamada, 1975; Timmer *et al.,* 2000).The incidence of melanose usually increases as trees age and the amount of dead wood in the canopy increases. If favorable conditions prevail, this disease may result in heavy economic losses.

There is a little information available on management of *Phomopsis citri* in Pakistan. There is a need to evaluate the bio-efficacy of available fungicides against the disease along with incorporation of cultural practices. Keeping all these aspects in view, present study was conducted with the objective of *In-vivo* evaluation of fungicides against *P. citri.*

Materials and methods

Site and plant selection

A field trail was conducted at a progressive farmer orchards of Toba Tek Singh (30° 58′ 16″ N, 72° 28′ 57″ E), Punjab during 2016-18. Citrus plant grown was Kinnow. Plants of the same age were selected for the experimental evaluation of chemicals.

Cultural practices

Diseased branches and leaves were pruned and burnt to lower the primary inoculum levels.

Preparation of Chemicals

Five treatments consisting of chemicals viz., Emesto Silver 240 FS % (Penflufen), Kocide® 3000 (Copper Hydroxide), Nativo 75% WG (Tebuconazole + Trifloxystrobin) and Score 250 EC (Difenoconazole) were prepared at recommended doses with three replications whereas in control treatment, plants were untreated.

Application of chemicals

Chemicals were evaluated for their efficacy against citrus melanose under in vivo conditions. Protectant spray was given in first week of March and treatments were continued at an interval of 15 days or if rainfall occurs till mid-April. For curative efficacy, chemicals were sprayed after appearance of pin head size spots. Spraying was done using manually operated high volume (knapsack) sprayer.

Data recording

Data was recorded on disease incidence (%) on leaves and fruits at weekly interval. Disease reduction over control (%) was measured by using the formula given below. There were five treatments and each treatment had five plants. All the treatments were replicated thrice in Randomized Completely Block Design (RCBD). Significance of fungicides was measured by

Student's T Test (Steel and Torrie, 1980).

$$\text{Disease incidence (\%)} = \frac{\text{Number of samples with melanose symptoms}}{\text{Total number of samples}} \times 100$$

$$\text{Diseases reduction over control H\%H} = \frac{\text{DI in control} - \text{DI in treatment}}{\text{DI in control}} \times 100$$

Results

In general, all the tested fungicides exhibited significant antifungal potential when applied as protectant while least effective or show no activity as curative. In protectant application, all the tested fungicides completely inhibited the infection and symptoms development till 2nd week (Fig 1).

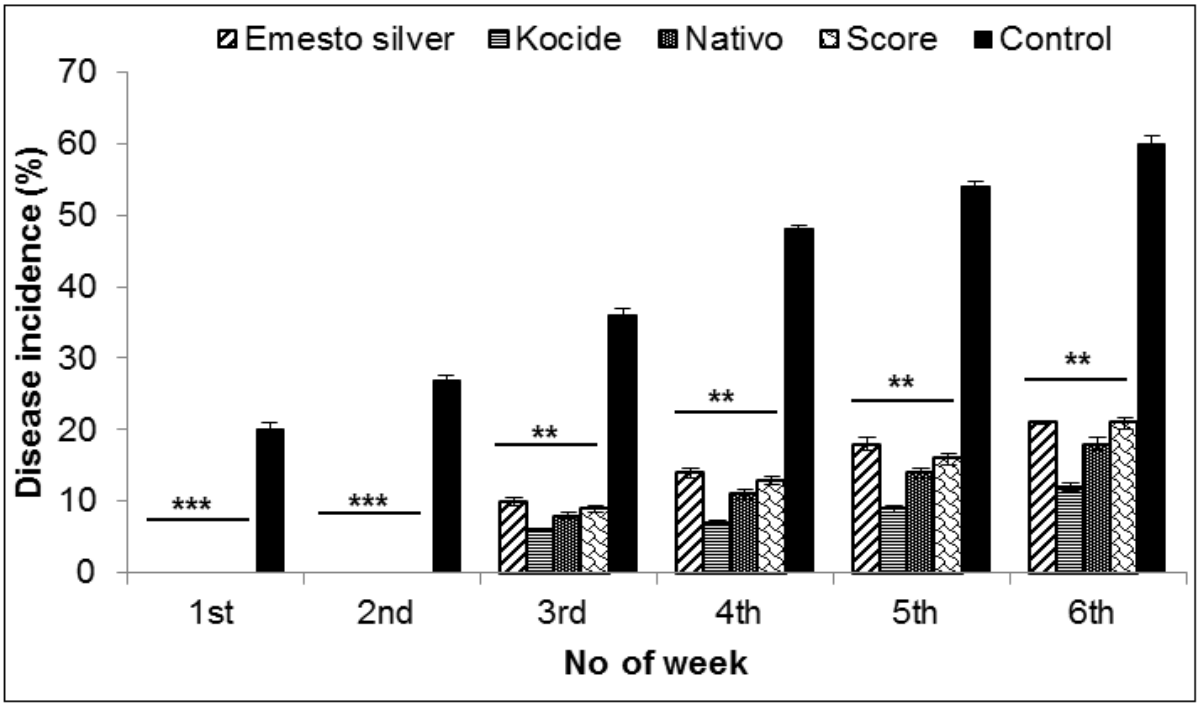

Fig. 1. Efficacy of different fungicides as protectant application against citrus melanose caused by *P. citri*. Vertical bars show standard errors of means of five replicates. Asterisk indicates statistically significant difference by Students' T-test. ** P < 0.01, ***P < 0.001.

Symptoms development was initiated in week 3 when environmental conditions were very favorable. The least disease incidence was observed with Kocide i.e., 12% followed by Nativo (18%) whereas the highest incidence (21%) was recorded by Score and Emesto silver on 6th week (Fig 1).

Disease reduction over control (%) was calculated and revealed that Kocide, Nativo, Score and Emesto silver significantly lowers (P < 0.01) the incidence of melanose by 80, 71, 65 and 65% respectively (Fig. 3).

In curative mode of application, all the tested chemicals showed non-significant difference among each other and symptoms development initiated from 1st week. Disease incidence ranged 45-52% as compared to the control i.e., 61% on 6th week (Fig. 2). Disease reduction over control (%) was measured after 6 weeks of curative application of fungicides as 25, 16, 18 and 13 % for Kocide, Nativo, Score and Emesto silver respectively (Fig. 3).

Discussion

The study was conducted during spring seasons of 2016-18 to evaluate the effectiveness of commercially available chemicals against citrus melanose. Overall all the tested chemicals were found effective as protectant when compared to untreated control. However, among the tested fungicidal chemicals, it was found that Kocide and Nativo were statistically significantly effective in reducing the disease incidence at recommended doses. Disease reduction

over control (%) was also measured significantly lowered in all chemical treatments than control where no chemical was applied. Percent disease reduction ranged between 65 to 80% as protectant application of fungicides whereas 13 to 25 % in curative fungicides.

These findings are in line with Bushing and Timmer (2000) who reported that protectant fungicides are more effective in controlling the citrus melanose than curative fungicides. They investigated pre and post infection efficacy of benomyl, febuconazole and azoxystrobin and found that when applied before inoculation, significantly reduced disease intensity whereas postinfection applications were only weakly effective. In another study, Whiteside (1977) described that benomyl is not highly effective when applied as a protectant, but it did reduce inoculum production. Zitko and Timmer (1997) investigated azole fungicides and reported reducedion in melanose when applied as a protectant. Strobilurin fungicide, and azoxystrobin were effective against melanose when applied as protectants in some previous studies (Timmer and Zitko, 1998; Zitko and Timmer, 1998).

In the United States only protectant copper fungicides are recommended for control of melanose (Knapp, 2000). Protectant fungicides, such as captan, chlorothalonil, and dithiocarbamates, have been registered for many years. Control of melanose requires several fungicide applications, beginning after bloom and continuing until mid-summer.

Even if symptom development is not prevented, sterol-biosynthesis-inhibiting (SBI) fungicides may prevent sporulation and, thus, reduce subsequent disease (Schwabe *et al.,* 1984; O'Leary and Sutton, 1986; Wilcox, 1990).

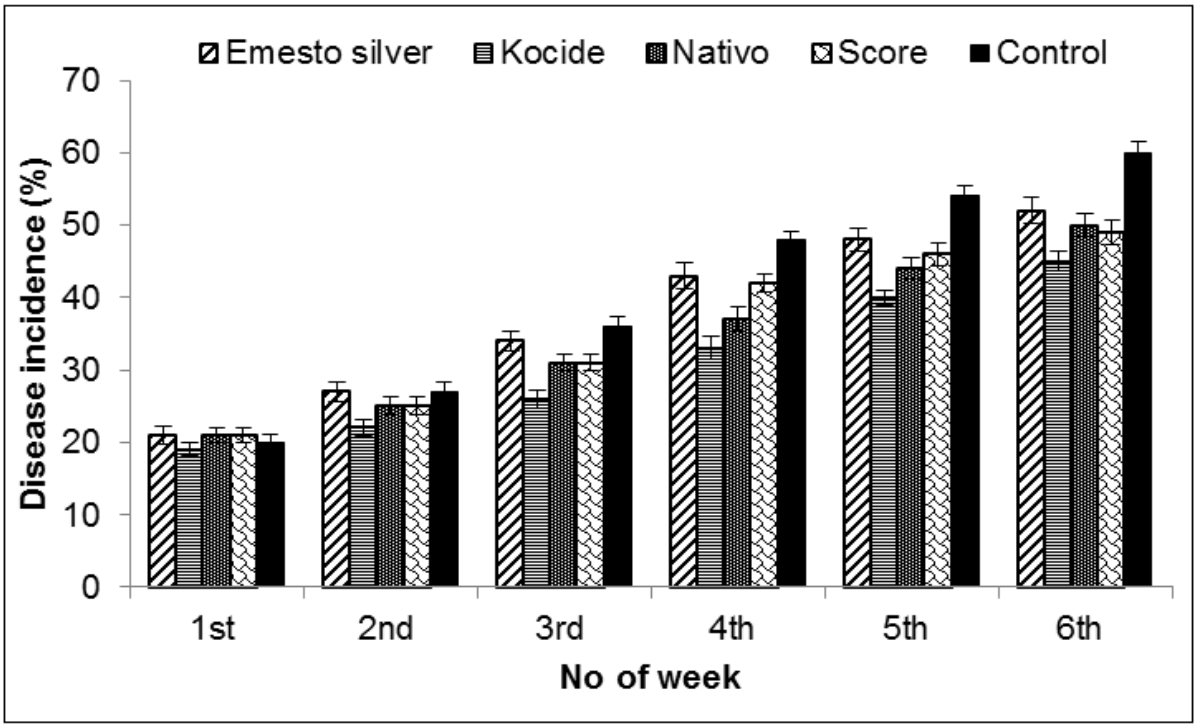

Fig. 2. Curative efficacy of different fungicides against citrus melanose caused by *P. citri*. Vertical bars show standard errors of means of five replicates.

It is evident from comparison of present and previous studies that melanose may be a difficult disease to control with post-infection sprays. *P. citri* can be re-isolated from lesions for only a few days after symptom development. Thus, even if a fungicide kills the pathogen in the early stages of infection, symptom development could proceed uninhibited. Copper fungicides may be used as protectants for citrus melanose control. The use of fungicides in the laboratory in comparison to field depends on its *in*

vitro efficacy at minimal, economically acceptable dosages and their proficient and rapid transport to the infection site. Kocide and Nativo proved effective fungicides when applied as protectant in the present study however none of the tested fungicides completely inhibit the *P. citri* growth under in field conditions.

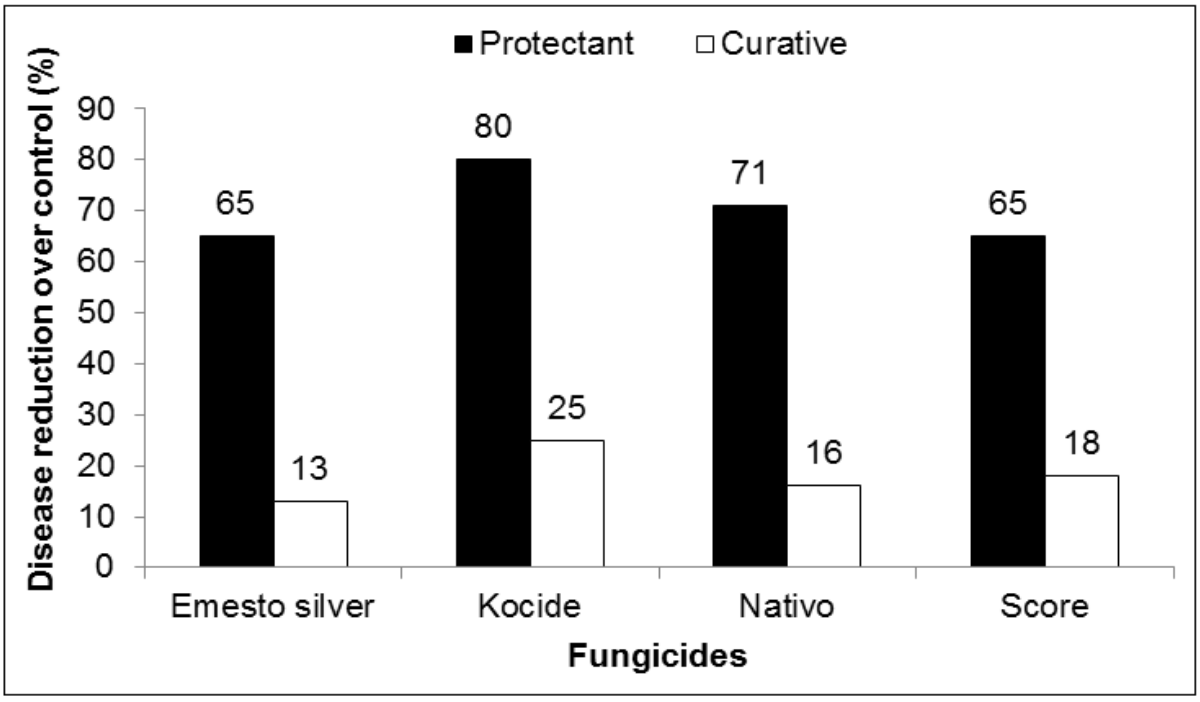

Fig. 3. Disease reduction over control (%) of fungicides when applied as protectant and curative against citrus melanose caused *P. citri*.

Conclusion

Melanose caused by *Phomopsis citri* may result in heavy economic losses if favorable conditions prevail. Present study was conducted to evaluate *In-vivo* Protectant and curative efficacy of fungicides against *P. citri*. Results revealed that all the tested fungicides exhibited significant antifungal potential when applied as protectant while least effective or show no activity as curative. It was concluded that Kocide is best for the management of citrus melanose when applied as protectant under in vivo conditions.

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