

Effectiveness of systemic and contact fungicides against *Alternaria citri* the causal organism of citrus brown spot disease in citrus mangroves of Pakistan

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ABSTRACT: Brown spot of citrus caused by *Alternaria citri* is among several diseases responsible for low crop production in Pakistan. Hence, the present study provides new information about the selection of chemicals for control of citrus brown spot in Pakistan. *A. citri* was isolated from different diseased samples of citrus plant collected from different orchards of Punjab Province of Pakistan. *In vitro* efficacy of fungicides was checked against *A. citri*. Fungicides viz; Thiophenate methyl, Difenconazole, hexaconazole, Metalaxyl + Mancozeb, Metiram, Mancozeb, Azoxystrobin, Dodine, Chlorothalonil, Fosetyl aluminium were evaluated against tested fungus at various concentrations (25, 50, 100, 200 and 400 ppm) by using poisoned food technique. The results showed that majority of the fungicides exhibited their highest inhibitory activity at a concentration of 400 ppm. Fosetyl aluminium, Metiram, Thiophenate methyl, Mancozeb and Metalaxyl + Mancozeb all exhibited maximum inhibitory mycelial growth of *A. citri* with 49.55, 52.96, 65.57, 84.86 and 100% inhibition, respectively at a concentration of 400 ppm whereas, a combination of Azoxystrobin and Difenconazole exhibited maximum inhibitory mycelial growth of *A. citri* up to 94.95% at fungicide concentration of 300 ppm. Chlorothalonil, Hexaconazole, Dodine, and Difenconazole were the most effective in inhibiting *A. citri* as no mycelial growth was observed at all concentrations and as such they produced 100% decreased in mycelia growth over the control. The study concluded that Fosetyl aluminium was the least effective fungicide against *A. citri*. The present study provides new information on the selection of chemicals for control of citrus brown spot in Pakistan.

Key words: Chemical control, disease incidence, disease severity, inhibition, mycelia growth.

INTRODUCTION

Citrus (*Citrus sinensis*) is an important horticultural crop in Pakistan contributing significantly to the Gross Domestic Products (GDP) and foreign exchange earnings for the nation (Haleem et al., 2005). Pakistan ranks 12th

worldwide in terms of citrus production with cultivation estimated to cover an area of 0.1922 million hectares with annual production of 2.213 million tons (FAO, 2013). The average yield of citrus is estimated at 12.7 tons per

hectare. Fruit production is predisposed to a number of factors amongst which insect/pest infestation is prominent that leads to low yield which in turn results in inferior quality. In addition, numerous other factors such as the development of diseases, non-certified nursery stock, post-harvest fruit losses and poor management practices contribute to the inferior quality of citrus fruits (Alferez et al., 2003). One prominent causative organism of brown spot of citrus is *Alternaria citri* (Pierce, 1902). Brown spot symptoms appear on leaf, twigs and fruits, with the characteristic symptom of this disease being circular to oval brown lesions, yellow halo development around the lesions because of toxin production by the pathogen (Dewdney and Timmer, 2012). At initial stages, foliar lesions are circular but having tails and surrounded by leaf vein which makes the lesion to appear as eyespot. The severity of infection depends upon the cultivar and favourable environmental conditions. The most pronounced symptoms can cause defoliation, wilting, dieback and premature fruit drop (Otani et al., 1995). The causal agent of citrus black rot is *Alternaria citri* which is the first citrus associated *Alternaria specie* (Pierce, 1902). On the basis of morphological similitude, the pathogen of brown spot of citrus was identified as *A. citri* (Doidge 1929, Ruehle 1937, Kiely 1964, Pegg 1996). Brown spot mostly infects tangerine and tangerine hybrids leading to fruit drop and reduced yield caused by *A. citri*. Conidia are produced on all parts of the plant that are susceptible to the disease. For the disease control in general, protectant fungicides are effective in inhibiting spore germination. However, this has been shown to be difficult in humid climate regions like that of Pakistan (Timmer et al., 2003). Nevertheless, chemical control remains the most effective control measure compared with other control strategies due to chemical post-infection activity and availability to growers (Bushong and Timmer, 2000). Chemical control (fungicide use) of the disease is the most common means worldwide and Tebuconazole, Chlorothalonil, Azoxystrobin, Thiophenate methyl, Fosetyl aluminium, and benomyl are some of the fungicides that considerably reduce the severity of brown spot (Timmer and Zitko, 1997). Copper-based fungicides give good results but there are disadvantages of copper as it darkens then blemishes and enhances the attack of lesion causing insects while it's accumulation in the soil becomes toxic to plants (Whiteside, 1983). Brown spot of citrus is among several diseases responsible for low citrus crop production in Pakistan thus complicating an already problematic situation of pathogen resistance emerging against different fungicides. Though some works have been carried out on the control of *A. citri* in Pakistan (Johnson, 2006, Fatima et al., 2009, Murtaza et al., 2012, Cevheri, 2017), insufficient research exists specifically on the control of *A. citri* in citrus plantations in Pakistan. It is at the backdrop of this that the current study was aimed at evaluating the effectiveness of systemic and contact fungicides against *A. citri*, the causal organism of citrus brown spot disease in citrus mangroves

of Pakistan.

MATERIAL AND METHODS

Varietal screening

In order to assess disease incidence and severity, a survey was conducted in areas of Khyber Pakhtunkhwa (KPK) and Punjab provinces of Pakistan. Different citrus varieties were selected from different orchards in each area. A total of 102 plants were randomly selected from the different orchards of KPK and Punjab provinces of Pakistan. A sample size of ten (10) plants and fifty (50) fruits were selected from each orchard of Citrus to record disease incidence and severity. Computations were achieved with the help of the following formulae (Safdar et al., 2010);

$$\text{Disease Incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100 \quad \dots \text{Eq. 1}$$

$$\text{Disease Severity (\%)} = \frac{\text{No. of infected fruits}}{\text{Total No. of fruits}} \times 100 \quad \dots \text{Eq. 2}$$

For assessing disease severity, the rating scale for assessment of leaf spot diseases of citrus as described by (Timmer and Zitko, 1997) (Table 1) was used.

Diseased specimen collection

Diseased samples were collected from randomly selected plants of 8 years old orchards to determine the infectious fungi associated with the diseases. The samples (one specific grape variety per location) were collected from twelve different areas (Table 2) resulting in a total of twelve (12) citrus varieties. Samples were aseptically collected and packed in sterile polythene bags and kept in the ice box and then transported to the diagnostic mycology laboratory of Department of Plant Pathology, University of Agriculture Peshawar, Pakistan for further processing within 2 hours after collection.

Isolation, purification and identification of pathogenic fungi

Diseased tissues from the samples were cut into small pieces (4 mm) and disinfected with 1% Chlorox solution and washed twice with distilled water and dried on blotter paper and placed on sterilized Petri plates containing potato dextrose agar (PDA) (Bio Basic Inc.) at 25°C with illumination of 600 Lux ((Ploetz and Gregory, 1992). Percentage colonization and relative density were calculated after 7 to 8 days of incubation. The isolated

Table 1. Disease rating scale of citrus brown spot.

Score	Description	Infection
0	No symptoms observed	No infection
1	25% leaf area covered with spots	Slight
2	26-50% leaf area covered with spots	Moderate
3	51-75% leaf area covered with spots	Severe
4	75% and above leaf area covered with spots	Very severe

Where 0, 1, 2, 3 and 4 means resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible respectively.

Table 2. Selected citrus varieties and locations of surveyed farms.

Item No	Citrus varieties	Location
1	Sour orange	Mardan
2	Mausambi	Nowshera
3	Kinnow	Sargodha
4	Coleoptera	Peshawar
5	Rio Red	Hazzara
6	Feuteral Early	Swabi
7	Mineola	Swat
8	Rangpur lime	Jhang
9	Faislabad pride	Mianwali
10	Foster	Sahiwal
11	Duncan	Gujranwala
12	Valencia late	Sialkot

fungi associated with diseased samples were purified by aseptically transferring unto fresh agar slants and incubating as before. For microscopic examination, fungi isolates were prepared by gently lifting the mycelia mat with a sterile inoculation pin into a drop of lactophenol blue on a slide, teased (spreading the mat) and covered with another slip and observed under a microscope. Fungi were identified by morphological characterization and the use of identification keys (Domsch et al. 1980; Samson et al., 1981; Samson et al., 1995).

In vitro* screening of fungicide against *Alternaria citri

The poisoned food technique described by Das et al. (2010) was employed in the *in vitro* test of the efficacy of ten selected fungicides (Table 3) against *A. citri*. The fungicides were tested against *A. citri* at different concentrations (25, 50, 100, 200 and 400 ppm) by combining appropriate quantities of the fungicides with an autoclaved PDA and mixing very well then plating in sterile Petri dishes and allowed to solidify. With the assistance of a sterile cork borer, a 5 mm mycelia disc of 72 hours old culture of *A. citri* was taken and aseptically placed in the center of the poisoned media. The experiment was

replicated thrice and plates containing only toxicated PDA was used as a control. Inoculated plates were incubated at 25°C ±2 for 7 days till the control plate attain growth of 9 cm. Percentage of inhibition was measured with the help of mean colony diameter and computed according to the formula (Eq. 3) (O'Rourke et al., 2009). Percent decrease in mycelial growth was calculated by comparing inhibition zones with respect to control treatment.

$$\text{Growth inhibition (\%)} = \frac{X-Y}{X} \times 100 \quad \dots \text{Eq. 3}$$

Where X = colony diameter in check and Y = colony diameter on fungicide treated plate.

Data analysis

All data recorded (triplicate) were subjected to analysis of variance (ANOVA) using SAS version 2002. Results are presented as means in tables analyzed and compared by applying Fisher's least significant difference at a confidence interval of 95%.

RESULTS

Disease severity and incidence

From the survey carried out on the brown spot disease incidence and severity as caused by *Alternaria citri* in different citrus fruits from citrus orchards of Panjabi province, results confirmed the presence of the disease in all orchards surveyed. However, the incidence and severity varied with location. Plate 1 depicts the diseased samples as they were picked from the orchard. The disease severity results are presented in Figure 1, indicating differences in severity of the disease among the citrus varieties correlating with the orchard locations (Table 2). Isolation of pathogen from diseased fruits revealed vividly that the pathogen responsible for the brown spot disease was *A. citri* as proven by pure cultures using morphological characterization and identification keys (Domsch et al., 1980; Samson et al., 1981; Samson et al., 1995) while Koch's postulate confirmed the pathogenicity of the isolates. Rangpur lime was the most

Table 3. List of fungicides used for *in vitro* screening test against (inhibition of) *A. citri* mycelial growth.

Trade Name	Active ingredient	Action	a.i (%)	Formulation	Toxicity class	Manufacturer
Score	Difenoconazole	S	250	SC	III	Syngenta
Topsin-M	Thiophenate Methyl	S	70	WP	III / IV	Arysta Life science
Dithane M-45	Mancozeb	C	80	WP	IV	Arysta Life science
Ellectus super	Azoxystrobin + Difenoconazole	C	25,8	SC	III	Evyol Group
Contaf Plus	Hexaconazole	S	5.1%	EC	I	Jaffer Group
Bayer Chlorothalonil	Chlorothalonil	S	500	SC	I	Bayer crop sciences
Alliete	FosetylAluminium	S	80	WG	III	Bayer crop sciences
Syllit	Dodine	C	400	SC	I	Collin Cambell Chemicals
Polyram DF	Metiram	S	80	WP	III	Swat agro
Ridomil Gold	Macozeb + Metalaxyly	S	68	WG	III	Syngenta

Where a.i, WP, EC, C, S, WG and SC mean active ingredient, wettable powder, emulsifiable concentrate, contact, systemic, wettable granules, and soluble concentrate respectively.

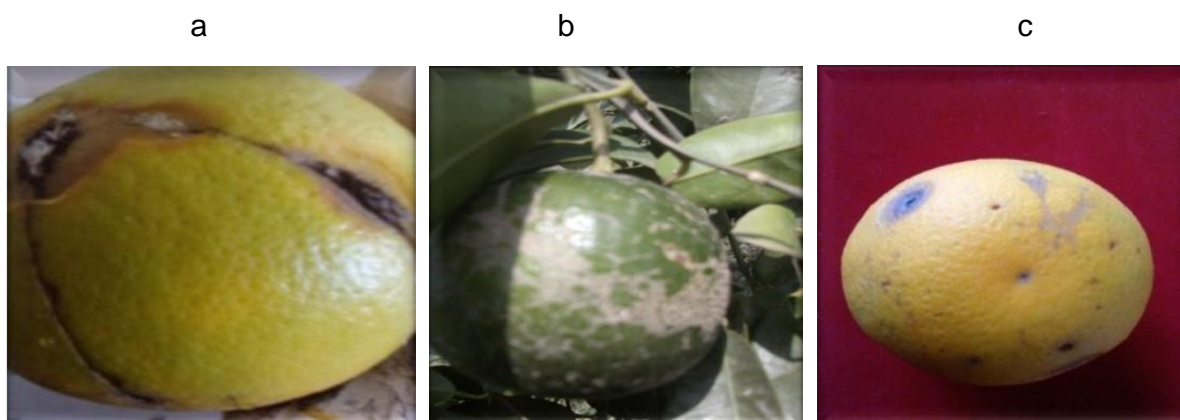


Plate 1. Pictures of fruits (a) Sour orange (b) Duncum and (c) Peach showing diseased condition as collected from the citrus orchards.

susceptible variety that was revealing significantly higher (56.0%) infection percentage while Faisalabad pride and Valencia late were relatively resistant to *A. citri* by showing significantly ($p < 0.05$) lower disease severity of 40.0 and 41.0%, respectively. Disease severity of 54.0, 53.0, 53.0 and 51.0% as revealed in Feuterall early, Rio red, Duncan and Coleoptera Riored respectively were not significantly ($p > 0.05$) different to Rangpur lime (Figure 1). Mausambi, Sour orange, Kinnow and Foster were averagely susceptible to the disease with 50.3, 50.0, 47.2 and 45.0% disease severities, respectively (Figure 1). Figure 2 depicts the results of the survey conducted to assess disease incidence of citrus orchards. There was evidence of disease incidence in all the orchards surveyed (Figure 2). The disease incidence ranged between 9.9 and 35.6% with the highest incidence associated with orchards at Sargodha (35.6%) and the least at Jhag (9.9%). More than

half (58.33%) of the orchards surveyed recorded diseases incidence of 20% and above.

Screening of fungicides against *A. citri*

The result of the study suggests that among the ten (10) fungicides evaluated against *A. citri*, interestingly, Dodine, Hexaconazole, and Chlorothalonil were most effective in controlling *A. citri* as they exhibited 100% inhibition at all concentrations (Table 4) except for Difenoconazole which was effective (100% inhibition) at lower fungicide concentrations (25, 30 and 50 ppm) while relatively less effective (65.8 and 58.0% inhibition) at higher fungicide concentrations (400 and 200 ppm) respectively. The results of Fosetyl aluminium were almost at par with Metiram with percent average decrease in colony growth

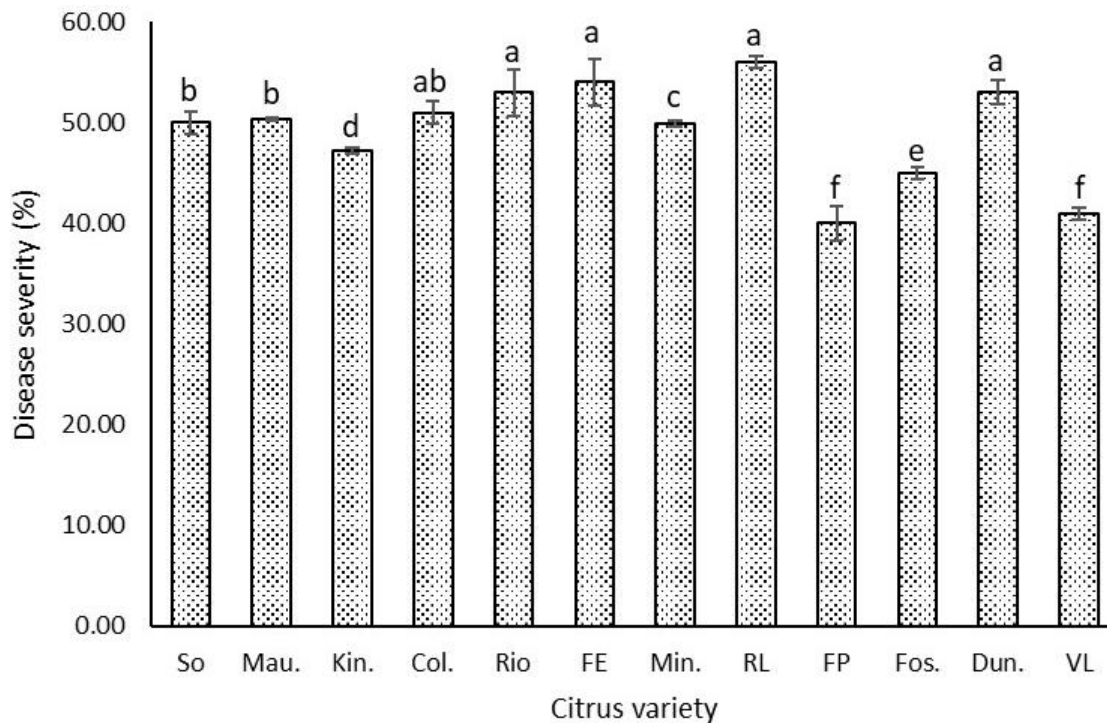


Figure 1. Disease severity due to *A. citri* on various citrus varieties. Where So, Mau., Kin., Col., Rio, FE, Min., RL, FP, Fos., Dun., and VL mean Sour orange, Mausambi, Kinnow, Coleoptera, Rio Red, Feuteral Early, Mineola, Rangpur lime, Faisalabad pride, Foster, Duncan, Valencia late citrus varieties respectively. Different letters indicate significant ($p < 0.05$) difference.

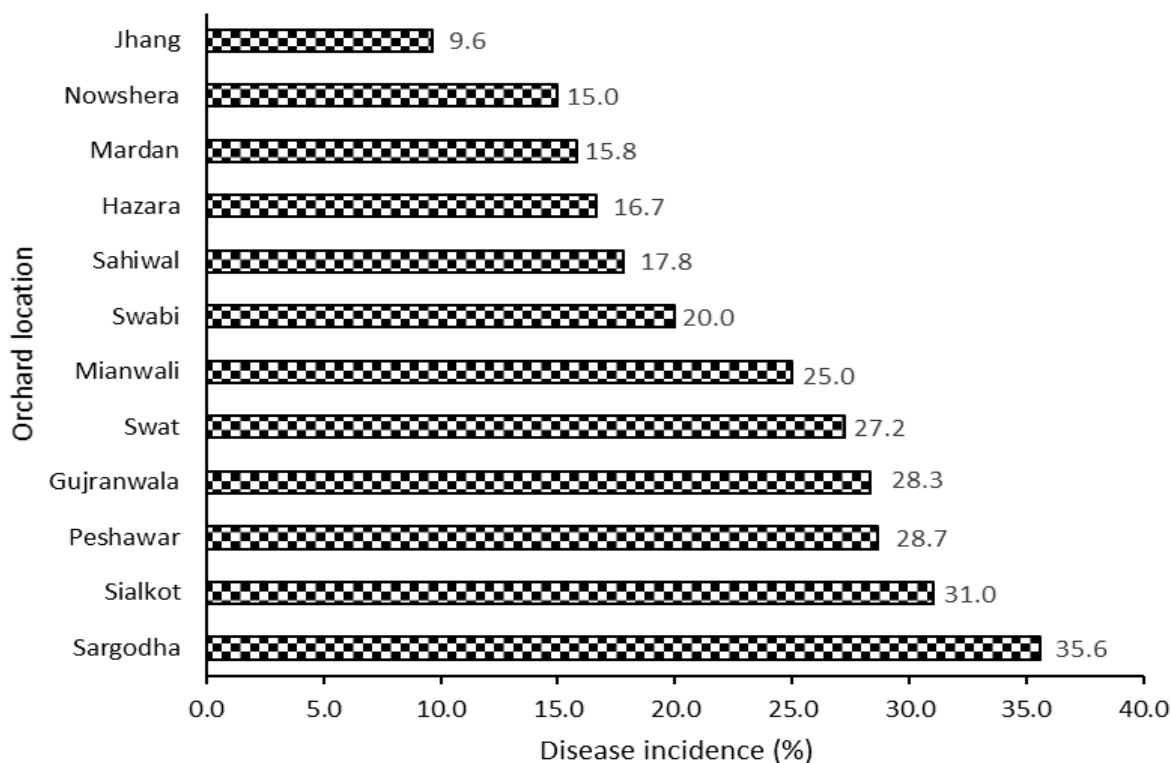


Figure 2. Disease incidence in citrus orchards of Khyber Pakhtunkhwa (KPK) and Punjab provinces of Pakistan.

Table 4. Average decrease in colony growth over control at different concentrations (%).

Fungicide	Concentration of fungicide (ppm)										
	25	30	50	75	100	120	200	240	300	400	600
Metalaxyl + Mancozeb	36.94	-	41.99	-	51.72	-	64.31	-	-	100.00	-
Fosetyl Aluminium	22.69	-	29.37	-	44.51	-	45.77	-	-	49.55	-
Metiram	29.38	-	39.84	-	45.39	-	51.19	-	-	52.96	-
Azoxystrobin + Difenconazole	-	-	-	-	-	91.17	92.05	-	94.57	-	-
Chlorothalonil	100.00	-	-	-	100.00	-	100.00	-	-	100.00	-
Thiophenate Methyl	41.61	-	47.61	-	52.96	-	54.00	-	-	58.23	-
Mancozeb	68.47	-	71.37	-	75.66	-	80.22	-	-	84.86	-
Difenconazole	-	100.00	100.00	100.00	-	-	58.00	-	-	65.77	-
Hexaconazole	-	-	-	-	-	-	-	100.00	-	100.00	100.00
Dodine	-	-	100.00	-	100.00	-	-	-	-	100.00	-

of 22.7, 29.4, 29.4, 39.8, as well as 44.5% and 45.4, 45.8, 51.2, 49.6 as well as 52.96% at concentrations of 25, 50, 100, 200 and 400 ppm respectively. Mancozeb exhibited high percentage decrease in colony by providing more than 65% growth inhibition at all concentrations (Table 4). In the case of a combination of Metalaxyl and Mancozeb, the result showed that there was relatively significant decrease in colony growth of *A. citri* over the control with 36.94, 41.99, 51.72, 64.31 and 100% *A. citri* colony growth inhibition at 25, 50, 100, 200 and 400 ppm concentrations respectively (Table 4). Mixture of Azoxystrobin and Difenconazole was also effective in controlling reproduction by inhibiting colony of the pathogen (*A. citri*) at 100, 200 and 400 ppm concentrations with average decrease in colony growth percentages being 91.17, 92.05 and 94.57 % respectively. On the other hand, the average decrease in colony growth percentages exhibited by Thiophenate Methyl were 41.61, 47.61, 52.96, 54 and 58.23% at 25, 50, 100, 200 and 400 ppm concentrations respectively (Table 4).

DISCUSSION

Citrus brown spot is an important disease of citrus present throughout the world and the pathogen which was isolated from disease samples was *Alternaria citri*. In the current study, it was found that *A. citri* was associated with spread of the citrus brown spot disease. The pathogenicity test of the current study revealed that *A. citri* was associated with the citrus brown spot disease development and 25 to 30°C was suitable temperature for growth and reproduction of conidia as proved by Timmer et al. (2000). Twelve varieties of citrus were evaluated in the study for their susceptibility to the citrus brown spot disease. Rang pur lime citrus variety was most susceptible to infection with 56% disease intensity or severity. While the other varieties were equally susceptible to the disease, some were relatively resistance by showing lower disease severity as in the case of Foster citrus variety. The high disease incidence recorded at

Sargodha, Sialkot and Peshawar could be attributed to the climatic factors and the soil condition. One significant observation was that the orchards which were near high ways had higher disease incidence. Similar observation was made by Safdar et al. (2010). In this experiment of fungicides evaluation against the disease, fungicides viz; Metalaxyl and mancozeb, Thiophenate methyl, fosetyl aluminium, metiram, mancozeb, Azoxystrobin and difenoconazole inhibited 68% decrease in colony growth of *A. citri*. The results are in agreement with Timmer and Zitko (1992) in which fosetyl aluminium, mancozeb, azoxystrobin were proved to be the best for management of the disease in citrus orchards. Results in this study revealed that all fungicides greatly inhibited the colony growth of citrus brown spot pathogen. Hexaconazole, difenoconazole belong to triazoles group of fungicides and exhibited 100% decrease in colony growth of pathogen over the control plate *in vitro*. Dodine, chlorothalonil also gave 100% control against the pathogen. These fungicides (chlorothalonil, dodine, hexaconazole, difenoconazole) were most effective at all concentrations and they totally suppressed growth of pathogen. While Mancozeb, Thiophenate methyl, Metiram, Fosetyl aluminium gave maximum inhibition at 400 ppm concentration, mixture of azoxystrobin and Difenconazole was also effective and inhibited growth at all concentrations but gave maximum result at 300 ppm. Timmer and Zitko (1997) used similar mixture for suppressing growth of citrus brown spot pathogen. In a similar study, Chlorothalonil an ethylene bithiodiocarbamates fungicide provided control against *A. citri* in brown spot lesions (Johnson et al., 2003). Various researches have also showed that the mycelia growth of *A. citri* could be inhibited by mancozeb at 400 ppm concentration (Khanzada et al., 2005; Jamadar and Lingaraju, 2011; Khanzada and Shah, 2012).

Elsewhere, the evaluation of various fungicides revealed that Azoxystrobin, chlorothalonil, hexaconazole, difenoconazole, dodine were highly effective against *Alternaria spp.* (Timmer et al., 2004). Bhatia et al. (2002) also evaluated mancozeb, metiram against the citrus

brown spot pathogen at 200 ppm concentration and proved that the pathogen is suppressed at this concentration *in vitro*. In view of all the previous studies cited above, the current results are indicative of higher pathogen inhibition as some of the fungicides exhibited growth inhibition of up to 100%. Banik et al. (1998) and Sales (2009) also proved that Difenoconazole was most effective in field disease management. Miles et al. (2005), conducted an experiment on field and evaluated fungicides against brown spot of mandarins and confirmed the causative agent to be *A. citri* in subtropical areas of Australia. Their results indicated among others that Strobilurens along with copper and Mancozeb were more effective in citrus fungi control which is in consonance with the current study.

Conclusion

This study evaluated the efficacy of selected fungicides at different concentrations in controlling citrus brown spot caused by *Alternaria citri*. The study has revealed that the selected fungicides (Cholorthalonil, Hexaconazole, Dodine and Difenoconazole) produced 100% decreased mycelia growth over the control, hence were most effective in inhibiting *Alternaria citri* while Fosetyl Aluminium was the least effective. Thus, these fungicides (Cholorthalonil, Hexaconazole, Dodine and Difenoconazole) have potential for use to control brown spot disease of citrus caused by *Alternaria citri* in Parkistan.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

- Alferez, F., Agusti, M., & Zacarias, L. (2003). Postharvest rind staining in Navel oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. *Postharvest Biology and Technology*, 28(1), 143-152.
- Bhatia, A., Tesoriero, A. & Timmer, L. (2002). Evaluation of fungicides for control of *Alternaria* brown spot on Murcotts, 2001. *Fungicide and Nematicide Tests (online) Report 57*: M06. DOI. 10.1094/FN57.
- Bushong, P., & Timmer, L. (2000). Evaluation of postinfection control of citrus scab and melanose with benomyl, fenbuconazole, and azoxystrobin. *Plant Disease*, 84(11), 1246-1249.
- Cevheri, C. (2017). Preliminary Study on the Antifungal and Antimicrobial Activities of Some Medicinal Plants of Turkey. *Journal of Biotechnology Research*, 3(11), 110-113.
- Das, K., Tiwari, R. & Shrivastava, D. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agents: current methods and future trends. *Journal Medicinal Plants Research*, 4(2), 104 -111.
- Dewdney, M., & Timmer, L. (2012). Florida Citrus Pest Management Guide: Citrus Scab. University of Florida. Factsheet PP146.
- Doidge, E. M. (1929). Some diseases of citrus prevalent in South Africa. *South African journal of science*, 26(12), 320-325.
- Domsch, K. H., Gams, W., & Anderson, T. H. (1980). Compendium of soil fungi. Volume 1, *Academic Press*, (London) Ltd.
- FAO, Food and Agriculture Organization (2013). Statistical Yearbook: World Food and Agriculture. Rome, FAO: 307.
- Fatima, N., Batoool, H., Sultana, V., Ara, J., & Ehteshamul-Haque, S. (2009). Prevalence of post-harvest rot of vegetables and fruits in Karachi, *Pakistan. Pak. J. Bot.*, 41(6), 3185-3190.
- Haleem, U., Mushtaq, K., Abbas, A., Sheikh, A., & Farooq, U. (2005). Estimation of Export Supply Function for Citrus Fruit in Pakistan [with Comments]. *The Pakistan Development Review*, Pp.659-672.
- Humber, R. A. (1997). Fungi: identification. Manual of techniques in insect pathology, *Elsevier*, 153-185.
- Jamadar, M., & Lingaraju, S. (2011). In vitro evaluation of fungicides, botanicals and bioagents against *Elsinoe ampelina*-An incitant of anthracnose of grapevine. *Karnataka Journal of Agricultural Sciences*, 24(2), 146-148.
- Johnson, D. A., Martin, M. & Cummings, T. F. (2003). Effect of chemical defoliation, irrigation water, and distance from the pivot on late blight tuber rot in center-pivot irrigated potatoes in the Columbia Basin. *Plant disease*, 87(8), 977-982.
- Johnson, G. (2006). Pakistan citrus industry challenges: Opportunities for Australia-Pakistan collaboration in research, development and extension. Pakistan: Citrus Industry Survey and Workshops.
- Khanzada, M., & Shah, G. S. (2012). In-vitro evaluation of fungicides, plant extracts and bio-controlagents against rice blast pathogen *magnaporthe oryzae* couch. *Pak. J. Bot.*, 44(5), 1775-1778.
- Khanzada, M. A., Lodhi, A. M., & Shahzad, S. (2005). Chemical control of *Lasiodiplodia theobromae*, the causal agent of mango decline in Sindh. *Pakistan Journal of Botany*, 37(4), 1023.
- Kiely, T. (1964). Brown spot of Emperor mandarin. *Agricultural Gazette of New South Wales*, 75, 854-856.
- McKinney, H. (1923). A new system of grading plant diseases. *J. Agric. Res*, 26(2), 195-218.
- Miles, A., Willingham, S., & Cooke, A. (2005). Field evaluation of a plant activator, captan, chlorothalonil, copper hydroxide, iprodione, mancozeb and strobilurins for the control of citrus brown spot of mandarin. *Australasian Plant Pathology*, 34(1), 63-71.
- Murtaza, A., Shafique, S., Anjum, T., & Shafique, S. (2012). In vitro control of *Alternaria citri* using antifungal potentials of *Trichoderma* species. *African Journal of Biotechnology*, 11(42), 9985-9992.
- O'Rourke, T. A., Scanlon, T. T., Ryan, M. H., Wade, L. J., McKay, A. C., Riley, I. T., Li, H., Sivasithamparam, K., & Barbetti, M. J. (2009). Severity of root rot in mature subterranean clover and associated fungal pathogens in the wheatbelt of Western Australia. *Crop and Pasture Science*, 60(1), 43-50.
- Otani, H., Kohmoto, K., & Kodama, M. (1995). *Alternaria* toxins and their effects on host plants. *Canadian Journal of Botany*, 73(S1), 453-458.
- Pegg, K. (1996). Studies of a strain of *Alternaria citri* Pierce, the causal organism of brown spot of Emperor mandarin. *Queensland Journal of Agricultural and Animal Sciences*, 23,

- 15-28.
- Pierce, N. B. (1902). Black rot of oranges. *Botanical Gazette*, 33(3), 234-235.
- Ploetz, R. C., & Gregory, N. (1992). Mango malformation in Florida: distribution of *Fusarium subglutinans* in affected trees, and relationships among strains within and among different orchards. *IV International Mango Symposium*, 341, 388-394.
- Ruehle, G. (1937). A strain of *Alternaria citri* Ellis and Pierce causing a leaf spot of rough lemon in Florida. *Phytopathology*, 27, 863-865.
- Safdar, A., Javed, N., Khan, S. A., Khan, H. U., Rehman, A., & Haq, I. (2010). Survey and investigation of different citrus growing areas for citrus sudden death syndrome. *Pak. J. Phytopathol*, 22(2), 71-78.
- Ploetz, R. C., & Gregory, N. (1992). Mango malformation in Florida: distribution of *Fusarium subglutinans* in affected trees, and relationships among strains within and among different orchards. *IV International Mango Symposium*, 341.
- Samson, R. A., Hoekstra, E. S., Frisvad, J. C., & Filtenborg, O. (1995). A Book Review: Introduction to Food-borne Fungi. *Dairy Food and Environmental Sanitation*, 15(7), 441-442.
- Samson, R. A., Hoekstra, E. S., Frisvad, C., & Fittenborg, C. (1995). Introduction to Food-borne Fungi. Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. *Wageningen, Ponsen and Looyen*, 33-112.
- Timmer, L., Darhower, H., Zitko, S., Peever, T., Ibanez, A., & Bushong, P. (2000). Environmental factors affecting the severity of *Alternaria* brown spot of citrus and their potential use in timing fungicide applications. *Plant Disease*, 84(6), 638-643.
- Timmer, L., Mondal, S., Peres, N., & Bhatia, A. (2004). Fungal diseases of fruit and foliage of citrus trees. *Diseases of Fruits and Vegetables*, 1, 191-227.
- Timmer, L., & Zitko, S. (1992). Evaluation of fungicides for control of citrus scab, 1991. *Fungicide and Nematicide Tests*, 47, 334.
- Timmer, L. & Zitko, S. (1997). Evaluation of fungicides for control of *Alternaria* brown spot and citrus scab. *Proceedings - Florida State Horticultural Society*, 110, 71-76.
- Timmer, L. W., Peever, T. L., Solel, Z., & Akimitsu, K. (2003). *Alternaria* diseases of citrus-novel pathosystems. *Phytopathologia Mediterranea*, 42(2), 99-112.
- Whiteside, J. (1983). Fungicidal effects of some acaricides on *Mycosphaerella citri*. *Plant disease*, 67(8), 864-866.