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Management of *Phytophthora* species associated with citrus decline in Pakistan

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Abstract
Different fungicides were evaluated against *Phytophthora* spp. Three concentrations 100, 200 and 300 ppm were used for each fungicide. Results showed that all fungicides significantly inhibited mycelial growth of *P. nicotianae* at 3rd, 5th and 7th day at different concentrations. Higher percent inhibition was given by Topsin-M, 58.3%, 56.4% and 51.3%, at 3rd, 5th and 7th day, respectively at concentrations 100-300 ppm. Least percent inhibition was given by Success 15.1%, 10.3% and 16.6%. Mycelial growth of *P. citrophthora* was also significantly inhibited by all fungicides at 3rd, 5th and 7th day at different concentrations. Topsin-M showed best results against *P. citrophthora* and inhibited mycelial growth to 71.7%, 66.6% and 60.4%, respectively at 3rd, 5th and 7th day. Minimum percent inhibition was given by Copper oxychloride 22%, 15.6% and 9.3%, and Kumulus 16.9%, 8.3% and 7.3% at 100-300 ppm concentrations at day's interval of 3, 5 and 7.

Keywords: citrus, disease, fungicide, evaluation, control

Introduction
Pakistan is bestowed with fertile land and favorable climatic conditions for citrus cultivation. Different varieties grown in Pakistan are kinnow, musambi, fruter early, sweet lime, grape fruit, pine apple and lemon etc. Among them, kinnow is at the top with 86% of the total citrus produced of the country and is followed by Musambi (10%), fruiter early (4%) and Red blood (1%) [14]. Citrus diseases that are produced by fungi, bacteria, viruses, nematode and spiroplasma are the main reason for lower yield. Among these diseases there are few important diseases of citrus that are caused by *Phytophthora* spp. [9]. There are several species of *Phytophthora* that are responsible for citrus diseases but most important are *Phytophthora citrophthora* and *Phytophthora nicotianae* that are responsible for damping of young seedling [2]. Gummosis is present in all citrus producing regions of the world and producing 10-30% losses every year. Combination of high temperature and humidity is suitable for disease development in citrus orchard. This disease mostly attacks the plant when scion comes in contact with soil or when irrigated water touches the scion [13].

It is need of time to manage these destructive plant pathogens to increase citrus production. There are several ways to manage plant diseases like use of resistant varieties, biological control agents, use of synthetic chemicals and integrated plant disease management. Among them, use of chemicals is most practised and easy way to manage plant diseases. There are many fungicides available in the market for this purpose. Fungicide like metalaxyl and mancozeb are effective to inhibit mycelial growth of *Phytophthora vignae* that causes root rot of cowpea [5]. Fosetyl-Al gives good control against *Phytophthora* root rot when applied as foliar spray, sleeve drench to seedling or trunk injection to mature tree [19]. Therefore, keeping in mind the devastating nature of pathogens, present study was designed to evaluate different chemicals to control *Phytophthora* spp. associated with citrus decline under laboratory conditions.

Materials and Methods
Present research was carried out during 2018-19 in Fungal Culture Bank and Plant Disease Diagnostic Laboratory of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha.

Collection of diseased samples
Surveys of different citrus orchards were conducted for the collection of diseased samples. From aerial portion, diseased leaves, shoots and fruits were collected. From root zone, rhizosphere soil as well as infected roots were collected.

Isolation of Pathogen
PARP media was used for isolation of pathogen from collected samples while potato dextrose agar (PDA) was used for multiplication of *Phytophthora* spp., and for evaluation of different fungicides, *Phytophthora* spp. were isolated by using two different isolation techniques.

Soil Plate method
Small amount of soil (0.005-0.015g) was taken and spread in petri plates having PARP media. Plates were incubated at 25ºC [1].

Tissue planting method
Infected roots were washed under tap water and converted into small segments of 3 mm. These segments were dipped into 70% ethanol for surface sterilization for 2-3 minutes and then washed with distilled water for two times. Five samples were placed in petri plates containing PARP media.

Plates were incubated at 25ºC in incubator and growth was observed after every 24 hours [8].

Identification

Fungi were identified on the basis of cultural and morphological characters of *Phytophthora* spp. like colony color, shape, size, hyphal morphology, morphology of sexual structure such as oospores, antheridia and oogonia and morphology of sporangium. These characters were compared with reported literature [3]. As sporangia do not produce on PDA, soil extract was prepared for the production of sporangia.

Evaluation of Fungicides

Preparation of stock solution

For each fungicide stock solution was separately prepared by dissolving 1g of fungicide in 999ml of distilled water. This stock solution was used for preparation of different concentrations.

Preparation of fungicidal concentrations

Three different concentrations (100, 200, 300 ppm) were prepared for each fungicide from 1000 ppm stock solution by using the formula:

$C_1V_1=C_2V_2$

$C_{1=}$Concentration of stock solution

$V_{1=}$ Volume of stock solution to be used

$C_{2=}$ Required concentration

$V_{2=}$ Volume of required concentration

Food Poisoning Technique

Food poison technique was use to check the efficacy of six different fungicides [6]. Fungicides used are given below in table 3.3. For each treatment three replicates were used. Potato dextrose agar was prepared and amended with fungicides before pouring. Three plates were poured for each concentration. After solidification, 5mm mycelial plug of fungi were placed in the centre of plates [7]. Plates were incubated at 25ºC and data were taken after 3 days, 5 days and 7 days. Percentage inhibition was measured by using the following formula:

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

Results

Evaluation of fungicides against *P. nicotianae*

Table 1: Fungicides used as treatment

Sr. No.	Fungicide	Active ingredient	Company name
1	Aliette	Fostyl-Al	Bayer
2	Topsin-M	Thiophanate Methyl	Arysta
3	Metalaxyl and Mancozeb	Metalaxyl and Mancozeb	Green Zone
4	Kumulus-DF	Sulfar	FMC
5	Copper oxychloride	Copper oxychloride	Capricorn
6	Success	Chlorothalonil and Metalaxyl	Arysta

Results clearly showed that all fungicides significantly inhibited mycelial growth of *P. nicotianae* at 3rd, 5th and 7th day ($P<0.05$). Higher percent inhibition 58.3%, 56.4% and 51.3% was given by Topsin-M at 3rd, 5th and 7th day, respectively followed by Metalaxyal+Mancozeb which inhibited mycelial growth to 54.3%, 29.1% and 40.6%. Least percent inhibition was given by Success and Copper Oxychloride and was 15.1%, 10.3% and 16.6%, and to 16.3%, 11% and 20.2%, respectively (Fig 1 (A).

At 3rd day after treatment, Topsin-M gave maximum percent inhibition at 100 and 200 ppm while Metalaxyal+Mancozeb, Success and Kumulus gave maximum inhibition at 300ppm. Highest percent inhibition 60%, 60% and 55% was given by Topsin-M followed by Metalaxyal+Mancozeb 52%, 52% and 58.7%, respectively. Lowest percent inhibition 2% was given by Success when 200 ppm concentration was used (Fig 1 (B).

Mycelial growth of *P. nicotianae* was significantly affected at 5th day at 100 ppm and 300 ppm. At 5th day, maximum percent inhibition 69.33% was given by Topsin-M at 300 ppm followed by same fungicide when used at 200 ppm (60.67%). Aliette gave maximum percent inhibition 37% at 100 ppm at 5th day which gradually decreased with increasing concentration. Metalaxyal+Mancozeb gave maximum 31.67% inhibition at 300 ppm at 5th day which decreased with decreasing concentration. Kumulus, Copper Oxychloride and Success were least effective fungicides (Fig 1 (C).

At 7th day after treatment, all fungicides significantly inhibited mycelial growth of *P. nicotianae* at 100 ppm, 200 ppm and 300 ppm. Maximum percent inhibition 69.33% was given by Topsin-M at 300 ppm while minimum percent inhibition was given by Success. Metalaxyal+Mancozeb and Kumulus gave maximum percent inhibition at higher concentration which decreased with decreasing concentration. Aliette gave maximum inhibition at 100 ppm which gradually decreased with increasing concentration (Fig 1 (D).

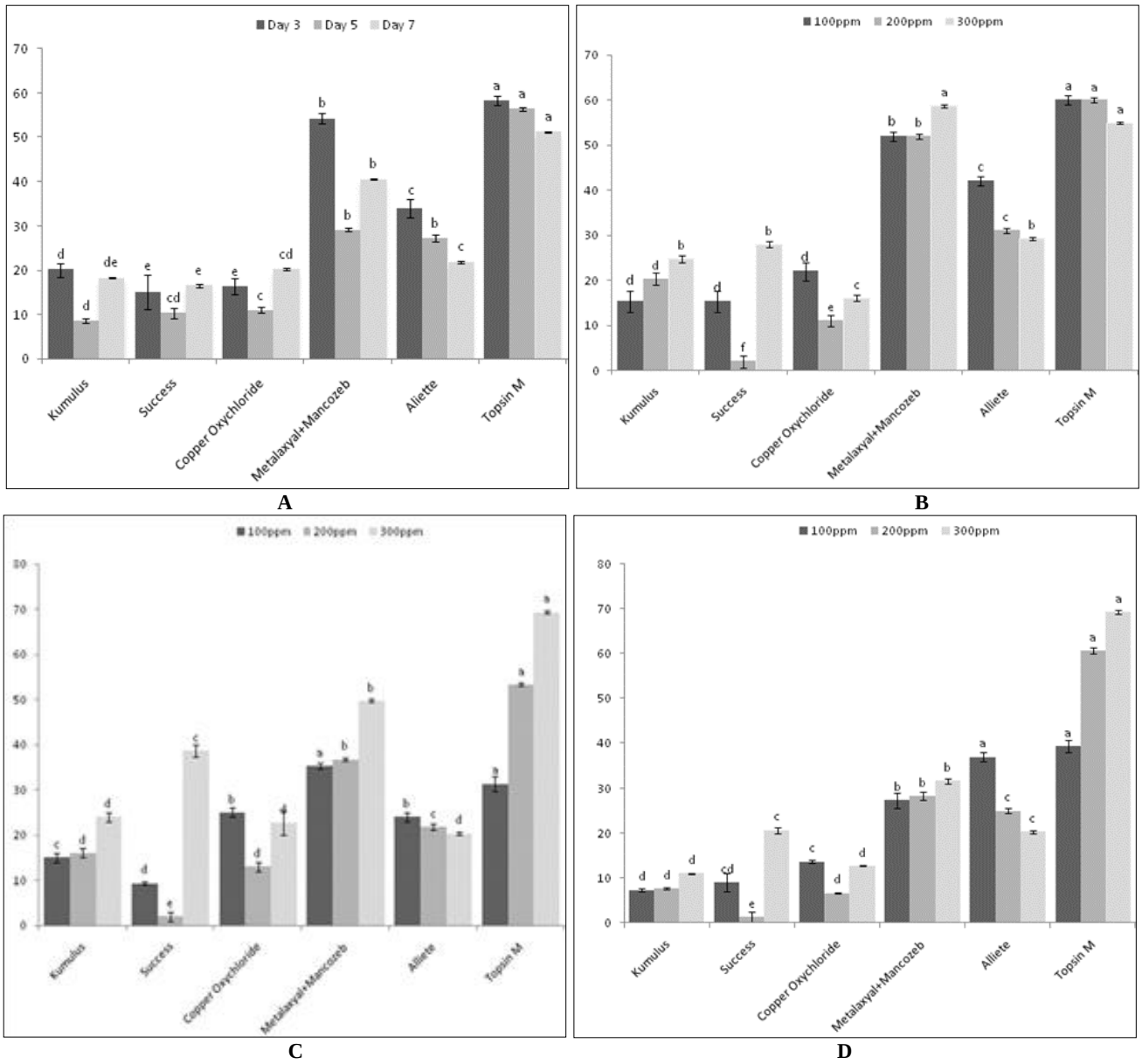

Fig 1: Evaluation of fungicide against *P. nicotianae* (A) Percent inhibition of mycelial growth at different time intervals (B) Percent inhibition of *P. nicotianae* at different concentrations after 3rd day after treatment (C) Percent inhibition of *P. nicotianae* at different concentrations after 5th day after treatment (D) Percent inhibition of *P. nicotianae* at different concentrations after 7th day after treatment

Evaluation of fungicides against *P. citrophthora*

Mycelial growth of *P. citrophthora* was significantly inhibited by all fungicides at 3rd, 5th and 7th days ($P<0.05$). Percent inhibition was higher after 3rd day of treatment and gradually decreased at 5th and 7th days in case of most fungicides except Aliette which gave best result after 5th day of treatment. Maximum percent inhibition was observed in case of Topsin-M, 71.7%, 66.6% and 60.4%, followed by Success, 64.9%, 58.2% and 45.9%, at 3rd, 5th and 7th days, respectively. Minimum percent inhibition was given by Copper Oxychloride 22%, 15.6% and 9.3%, and Kumulus 16.9%, 8.3% and 7.3% which was higher at 3rd day and decreased with increasing time interval (Fig 2A).

At 3rd day after treatment, maximum percent inhibition was observed at 300 ppm which gradually decreased with decreasing concentration. Topsin- M was most effective fungicide as it gave higher percent inhibition 87.3% at 300 ppm followed by Success which gave 73.3% mycelial inhibition at the same concentration. Copper Oxychloride and Kumulus were least effective fungicides and gave inhibition to 13.3%, 17.3% and 35.3%, and 15.3%, 16.3% and 19.6%, respectively (Fig 2 (B). At 5th day after treatment, all fungicides were effective at higher concentrations but their efficacy gradually decreased with decreasing concentration except Aliette which was most effective at lower concentration. Maximum percent inhibition was given by Topsin-M, 80.7% at 300 ppm, followed by Success which gave 71.7% at the same concentration. Kumulus and Copper Oxychloride were least effective. Minimum percent inhibition, 6.3% at 5thday, was given by Kumulus when 100 ppm concentration was used (Fig 2 (C).

At 7th day after treatment, higher percent inhibition 79.7% was observed in case of Topsin-M at 300 ppm followed by the same fungicide 63.5% when used @ 200 ppm. Success was second best fungicide which gave 34%, 42.3% and 63% at 100, 200 and 300 ppm, respectively. Kumulus was least effective fungicide in this study which gave 4%, 8.7% and 9.3% inhibition at 100, 200 and 300 ppm, respectively. Copper Oxychloride gave same results (7.3%) at 100 and 200 ppm while percent inhibition increased at 300 ppm (14%) (Fig 2 (D).

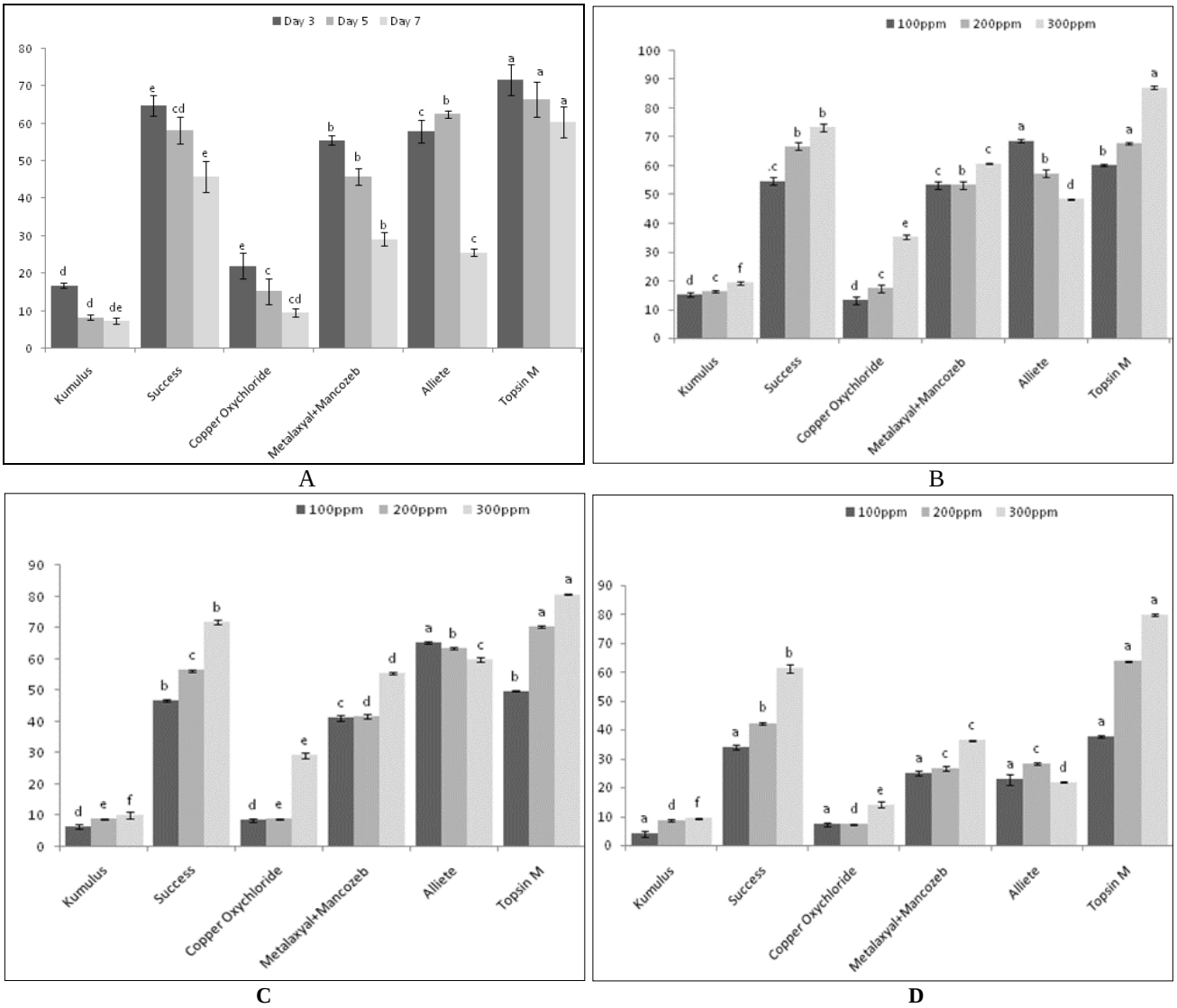

Fig 2: Evaluation of fungicide against *P. citrophthora* (A) Percent inhibition of mycelial growth at different time intervals (B) Percent inhibition of *P. citrophthora* at different concentrations after 3rd day after treatment (C) Percent inhibition of *P. citrophthor* at different concentrations after 5th day after treatment (D) Percent inhibition of *P. citrophthora* at different concentrations after 7th day after treatment

Discussion

Phytophthora diseases are serious threat to citrus groves. *P. nicotianae* and *P. citrophthra* are two most important pathogens of citrus diseases. Citrus gummosis, root rot and brown rot of fruit are diseases caused by these species. Gummosis is present in all citrus producing regions of the world and producing 10-30% losses every year [13]. These figures show that *Phytophthora* is one of the most damaging plant pathogen. There are several characteristics of *Phytophthora* which makes it worst plant enemy. These characters include its ability to spread through water and air, rapid production of inoculum, production of motile zoospores, production of chlamydospores and oospores for its survival outside plant tissues, wide host range, availability of host plant all around the year, ability of single species to cause diseases in multiple hosts and ability to cause more than one disease in single host. Without managing *Phytophthora* diseases it is impossible to get maximum yield in citrus.

In this study food poisoning technique was used for *in-vitro* evaluation of different fungicides at different concentrations. This is simple and easy technique for *in vitro* evaluation [17]. By using food poison technique, best fungicide and its best concentration against fungal pathogen can be tested in short time with limited resources [10]. In past many researchers used this technique to check the efficacy of different fungicides against fungal pathogens [11, 4].

In current research, efficacy of different fungicides was different at different concentrations and time intervals. Among tested fungicides, Topsin-M (thiophanate methyl) gave higher percent inhibition against both species. Topsin-M is broad spectrum systemic fungicide and has been found very effective against different fungi in previous studies [10]. Rather *et al*. [15] evaluated ten different fungicide including thiophanate methyl against soil borne fungi using food poisoning technique. They reported that thiophanate methyl significantly inhibited mycelial growth of *Phytophthora* spp. and other soil borne fungi. They further noted that efficacy of thiophanate methyl increased with increasing concentration. Sahi *et al*. [16] used food poisoning technique to check the efficacy of different fungicides against soil born fungus, *Botryodiplodia theobromae*, that causes quick decline of mango. They reported that among the tested fungicides, Topsin-M was the most effective fungicide at higher concentrations. They also reported that Topsin-M efficacy increased with increasing time intervals. Sultana

and Ghaffar [17] conducted *in vitro* experiments to evaluate the efficacy of fungicides, microbial antagonists and oil cakes against *Fusarium oxysporum*, the causal organism of root and seed rot of cucumber and bottle gourd. They reported that at 1000 ppm concentration of Topsin-M completely inhibited the mycelial growth of fungus. Hassan *et al*. [10] used food poisoning technique to check the efficacy of different fungicides against *Ilyonectria radicicola*, a soil borne fungus. They reported that Topsin-M affected the mycelial growth and sporulation of fungus and its efficacy increased with increasing concentration.

In present study, Metalaxyal+Mancozeb were the second best fungicide against *P. nicotianae* and *P. citrophthora*. It is systemic and protective fungicide and gives good control against Oomycetes. This fungicide significantly inhibited the mycelial growth of both species of *Phytophthora* at different concentrations and mycelial growth inhibition increased with increasing concentration. These results are similar to the studies conducted previously [4, 18]. Metalaxyal+Mancozeb have been found effective against wilt disease of pepper (Rather *et al*. [15]. They concluded that Metalaxyal+ Mancozeb significantly suppresses the mycelial growth of *Phytophthora* spp. and other fungal pathogens associated with wilt complex. It has also been noted against different fungal pathogens that efficacy of this fungicide increases with increasing concentration. Elshahawy *et al*. [4] conducted *in vitro* experiments by using food poisoning technique to check the compatibility of *Trichoderma* spp. with metalaxyal+mancozeb and six other fungicides to control soil borne fungi. They reported that metalaxyal+mancozeb was compatible with *Trichoderma* spp. and significantly inhibited soil borne fungi. Türkölmez and Dervis, [18] checked the activity of metalaxyal+mancozeb and other fungicides against *Phytophthora palmivora* that causes root rot of cherry and apricot. They reported that this fungicide significantly suppressed *P. Palmivora* and it was more effective at higher concentrations. Results of present study also indicated that Aliette (Fosetyl-al) significantly inhibited the mycelial growth of *P. nicotianae* and *P. citrophthora*. This fungicide has multiple modes of actions and gives two way systemic protections as it travels upward and downward in plant system. This fungicide not only stimulates plant defence system but also inhibits sporulation. Results of this study are similar to the study conducted by [11, 12]. Khanzada and Shah, [11] evaluated different fungicide against pathogen of rice blast disease. Results of their study showed that Aliette has ability to inhibit the mycelial growth of rice blast fungus. Moradi *et al*. [12] conducted experiment to evaluate the efficacy of Aliette against causal organism of pistachio gummosis. They reported significant control of pistachio gummosis with this fungicide.

Conclusion

Results of present study showed that evaluated fungicides are effective against *P. nicotianae* and *P. citrophthora* at concentrations of 100, 200 and 300 ppm. However, their efficacy varies with day's interval. Fungicide Topsin-M found most effective against both species.

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Conflict of interests

"The author(s) declare(s) that there is no conflict of interests regarding the publication of this article"

References

1. Arshi HA, Nasreen S. Isolation and identification of fungi from soil samples of different sites in Aurangabad city, India. International Journal of Science and Research. 2016; 5:419.
2. Drenth A, Guest DI. Diversity and management of Phytophthora in Southeast Asia. Australian Centre for International Agricultural Research (ACIAR), 2004.
3. Drenth A, Sendall B. Practical guide to detection and identification of Phytophthora. CRC for Tropical Plant Protection, Brisbane, Australia. 2001; 3:1-41.
4. Elshahawy IE, Haggag KH, Abd-El-Khair H. Compatibility of Trichoderma spp. with seven chemical fungicides used in the control of soil borne plant pathogens. Research Journal of Pharmaceutical Biological and Chemical Sciences. 2016; 7:1772-1785.
5. Fernando WGD, Linderman RG. Chemical control of stem and root rot of cowpea caused by *Phytophthora vignae*. Plant Disease. 1994; 78:967-971.
6. Fitsum S, Amin M, Selvaraj T, Alemayehu A. *In vitro* evaluation of some fungicides and bio-agents against common bean anthracnose (*Colletotrichum lindemuthianum* Sacc. Magnus) Briosi Cavara. African Journal of Microbiology Research. 2014; 8:2000-2005.
7. Gautam C, Prabhu HV, Nargund VB. *In vitro* Evaluation of fungicides, botanicals and bioagents against Peziotrichum corticolum causing black banded disease of mango. International Journal of Current Microbiology and Applied Sciences. 2017; 6:652-661.
8. Ghosh A, Shamsi S. Fungal diseases of rose plant in Bangladesh. Journal of Bangladesh Academy of Sciences. 2014; 38:225-233
9. Gopal K, Lakshmi LM, Sarada G, Nagalakshmi T, Sankar TG, Gopi V, *et al*. Citrus melanose (*Diaporthe citri* Wolf). A Review. International Journal of Current Microbiology and Applied Sciences. 2014; 3:113-124.
10. Hassan WA, Haleem RA, Saido KA. Chemical control of black foot disease caused by Ilyonectria radicicola on grapevine cuttings. Mesopotamia Journal of Agriculture. 2013; 41:448-56.
11. Khanzada M, Shah GS. In-vitro evaluation of fungicides, plant extracts and bio-controlagents against rice blast pathogen Magnaporthe oryzae couch. Pakistan Journal of Botany. 2012; 44:1775-1778.
12. Moradi M, Mohammadi AH, Haghdel M. Efficiency of Elite Fungicide for Control of Pistachio Gummosis. Journal of Nuts, 2017; 8:11-20.
13. Mounde LG, Ateka EM, Kihurani AW, Wasilwa L, Thuranira EG. Occurrence and distribution of citrus gummosis (*Phytophthora* spp.) in Kenya. African Journal of Horticultural Science, 2009, 2.
14. Naseer M. Report on kinnow export of Pakistan. Trade development authority of Pakistan, Government of Pakistan, 2010.
15. Rather TR, Razdan VK, Tewari AK, Shanaz E, Bhat ZA, Hassan MG, *et al*. Integrated management of wilt

complex disease in bell pepper (*Capsicum annuum* L.). Journal of Agricultural Science. 2012; 4:141.
16. Sahi ST, Habib A, Ghazanfar MU, Badar A. *In vitro* evaluation of different fungicides and plant extracts against Botryodiplodia theobromae, the causal agent of quick decline of mango. Pakistan Journal of Phytopathology. 2012; 24:137-142.
17. Sultana N, Ghaffar A. Effect of fungicides, microbial antagonists and oil cakes in the control of Fusarium oxysporum, the cause of seed rot and root infection of bottle gourd and cucumber. Pakistan Journal of Botany. 2013; 46:2149-2156.
18. Türkölmez Ş, Dervi ŞS. Activity of metalaxyl-m+ mancozeb, fosetyl-al, and phosphorous acid against *Phytophthora* crown and root rot of apricot and cherry caused by *Phytophthora palmivora*. Plant Protection Science. 2017; 53:216-225.
19. Young HS, Tepper BL, Mercer RT, Pelizzo G, Anelich R. Fosetyl-AI, *et al*. A Management Tool for Control of *Phytophthora* Root Rot. World Second Avocado Congress. 1992, 69-74.