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Evaluation and Determination of Minimum Inhibitory Concentration of Fungicides against Root Rot Pathogen of Citrus (*Phytophthora nicotianae*)

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ABSTRACT: *Phytophthora* species are one of the most devastating oomycetes causing root rot of citrus. The pathogen was earlier controlled by metalaxyl (phenylamide), but from 1980's, development of resistance to phenylamides have been reported. In the present study, efficacy of ten different fungicides, (7 systemic, 2 contact + systemic and 1 contact fungicides) was evaluated against the mycelial growth of *Phytophthora nicotianae* by poisoned food technique. The result revealed that all the tested fungicides were significant in controlling the mycelial growth as compared to control. Metiram 44% + Dimethomorph 9%, Metalaxyl 4% + Mancozeb 64%, Mandipropamid 23.4%, Fluopicolide 39.5%, and Fluopicolide (5.53% w/w) + Propamocarb hydrochloride (55.3% w/w) exhibited complete inhibition at 250, 500 and 1000 ppm. The least mycelial inhibition was exhibited by cyazofamide (33.85%, 43.85% and 62.31% at 250, 500 and 1000 ppm respectively. To study the minimum inhibitory concentrations (MIC's) of fungicides, the concentrations were increased/decreased. The response of fungicides on mycelial growth of *P. nicotianae* was highly variable. Based on MIC's, the fungicides were grouped into three groups (Group I, II and III). The MIC of Metiram 44% + Dimethomorph 9% was 10 ppm, followed by Metalaxyl 4% + Mancozeb 64%, Mandipropamid 23.4%, Fluopicolide 39.5%, and Fluopicolide (5.53% w/w) + Propamocarb hydrochloride (55.3% w/w) was 30 ppm and were placed in Group I. The MIC of Fosetyl Al, Chlorothalonil 50% + Metalaxyl 3.75% SC and Phosphorous acid was 1000 ppm and was placed in Group II. Azoxystrobin 20% + Difenoconazole 12.5% SC (2000 ppm) and cyazofamide (8000 ppm) were placed in Group III.

Keywords: Citrus, Root-rot, *Phytophthora nicotianae*, Fungicides, *in-vitro* evaluation, Dimethomorph.

INTRODUCTION

Phytophthora diseases are one of the most devastating oomycete plant pathogens, responsible for decline and short life span of citrus orchards throughout India (Naqvi, 2004; Das *et al*., 2011). Among these, *P. nicotianae* and *P. palmivora* are the most prevalent oomycetes causing decline of citrus orchards. The infection starts with the discoloration and browning of feeder roots, which ultimately affect the nutrient and water uptake and leading to decline of trees. Later, *Phytophthora* sp. becomes difficult to manage due to their long-term survival in the form of oospores in the soil, wider host range and long-distance dissemination.

The economic losses caused by *P. nicotianae* are very difficult to estimate, because of the wide host range and ecological niches. *Phytophthora nicotianae* are better adapted to abiotic stresses, especially to climate warming (Panabieres *et al.*, 2016). Therefore, this pathogen poses a continuous challenge to plant disease management programmes, which mostly rely on the use of chemicals. Traditionally, two fungicides belonging to FRAC group 4 *i.e.,* phenylamides (metalaxyl/mefanoxam) and FRAC group 33 *i.e.,* phosphonates (fosetyl-Al, potassium phosphite) were used, but from 1980's, development of resistance to phenylamides have been reported in *P. citricola, P. cryptogea, P. infestans, P. megasperma* and *P. nicotianae* (Davidse *et al.*, 1981; Shew, 1985; Ferrin and Kabashima 1991; Gisi and Cohen 1996; Hwang and Benson 2005; Stack and Millar 1985; Parra and Ristaino 2001; Jeffers *et al*., 2004; Taylor *et al*., 2006; Hu *et al.*, 2008). Though, the resistance to phosphonates is developed less frequently, development of phosphonate resistant strains of *P. capsici, P. cinnamomi*, and *P. infestans* had been reported (Cohen and Samoucha 1984; Veena *et al.*, 2010; Wilkinson *et al*., 2001).

The knowledge regarding fungicides with different mode of actions to manage metalaxyl resistant strains is very low which can be targeted by rotation of fungicides with different modes. The present study

focused on evaluating ten different fungicides (with different mode of action, target, and low-risk) under *in-vitro* conditions by poisoned food technique and developing practical solutions to control root rot disease of Citrus caused by *P. nicotianae*.

MATERIAL AND METHODS

Isolation of *P. Nicotianae.* The infected root samples were collected from a root rot and gummosis infected Nagpur mandarin (*Citrus reticulata*) tree situated at ICAR-CCRI farm site, Nagpur (40.829386'N, 77.845798). For *Phytophthora* isolation, 1-2 cm pieces were excised from infected root and surface sterilized using 2% sodium hypochlorite for 1 min, followed by 3 consecutive washes with sterile distilled water. The cut bits were further isolated on selective media *i.e*., Corn Meal Agar (CMA) medium amended with Pimiracin (0.01 g/L), Ampicillin (0.025g/L), Rifamycin (0.012g/L), Pentachlorobenzene (0.01g/L) and Hymexazol (0.08g/L) (Kannwischer and Mitchell 1978) and were incubated in the dark at 25°C for 4 to 5 days. The *Phytophthora* isolate was confirmed morphologically by asexual and sexual characteristics and molecularly by internal transcribed spacer region restriction fragment length polymorphism (ITS-RFLP) and Sanger sequencing of ITS region.

Pathogenicity assay was conducted in laboratory using floating disc of rough lemon (*C. jambhiri*) (Nath, 2013). The 15-20 mycelial discs from actively advancing margin of cultures, were placed in sterile distilled water to induce development of sporangia. The plates were incubated under continuous light at 25°C for 4 to 5 days. The zoospores were liberated from matured sporangia by giving cold shock. Leaves of rough lemon were further floated in zoospore suspension; the leaves were examined visually for disease symptom.

Evaluation of fungicides. Commercially formulated ten different fungicides (7 systemic, 1 contact and 2 contact + systemic) were evaluated *in vitro* by poisoned food technique for their efficacy in controlling root rot pathogen of Citrus, *Phytophthora nicotianae* (Nene and Thapiyal 2002). The details of fungicides are given in Table 1. Initially, the efficacy of systemic fungicides was tested at 250 ppm, 500 ppm and 1000 ppm and that of contact fungicides at 1000 ppm, 2000 ppm and 3000 ppm. The systemic + contact fungicides were evaluated at 250 ppm, 500 ppm, 1000 ppm, 2000 ppm and 3000 ppm (Thomas and Naik 2017). Further, the concentrations of fungicides were decreased or increased to determine the MIC values of fungicides (if no growth was observed in the lowest concentration, the concentration was reduced whereas, if growth was observed in highest concentration, the concentration was increased).

Fungicide stocks were prepared in de-ionised water and were added separately to molten CMA medium, to obtain desired concentrations. Five mm mycelial disc of actively growing 7 days old culture was placed in the centre. Plate without any fungicide was maintained as control. Three replications were maintained for each concentration. All the plates were incubated at 25±1ºC temperature for 10 days (Thomas and Naik 2017).

Table 1: List of common name, chemical name, class, and mode of action of the fungicides evaluated in the study.

Sr. No.	Trade Name	Active Ingredient	Fungicidal class	Mode of action
1.	Ridomil Gold	Metalaxyl 4% + Mancozeb 64%	Phenylamides and dithiocarbamates	Systemic
2.	Alliette	Fosetyl Al	Phosphonate	Systemic
3.	Amistar Top	Azoxystrobin 20% + Difenoconazole 12.5% SC	QoI fungicides	Systemic
4.	Ranman	Cyazofamide	Qil fungicides	Systemic
5.	Prophyt	Phosphorous Acid	Phosphonate	Systemic
6.	Profiler	Fluopicolide 39.5%	Benzamide	Systemic
7.	Acrobat	Metiram 44% + Dimethomorph 9%	Cinnamic acid	Systemic
8.	Revus	Mandipropamid 23.4%	Cinnamic acid	Contact
9.	Infinito	Fluopicolide (5.53% w/w) + Propamocarb hydrochloride (55.3% w/w)	Carbamate	Contact and systemic
10.	Folio Gold	Chlorothalonil 50% + Metalaxyl 3.75% SC	Chlorothalonil	Contact and systemic

The Percent inhibition by fungicides is worked out by following formula:

Percent inhibition = $\frac{C-T}{C} \times 100$

Where, C = colony diameter (mm) of the control

T = colony diameter (mm) of the test plate (Gupta and Tripathi 2011).

Statistical analysis. All data was statistically analysed using analysis of variance (ANOVA) at probability level 0.05 by using software package OPSTAT (Sheoran *et al.*, 1988).

RESULTS

Isolation and identification of *P. nicotianae.* *Phytophthora nicotianae* isolate, CPhy-38 was isolated from infected roots of a Nagpur mandarin tree. The identity of isolate was confirmed by ITS sequencing (submitted to Genbank under the accession number OM758322) and RFLP analysis of the amplicon (~ 900 bp) generated after amplification of ITS6/4 product of the isolate. The banding pattern of 400 bp, and 120 bp was obtained upon restriction digestion by *Msp*I and 745, 117, 52 by *Alu*I which is typical of *P. nicotianae* (Fig. 1).

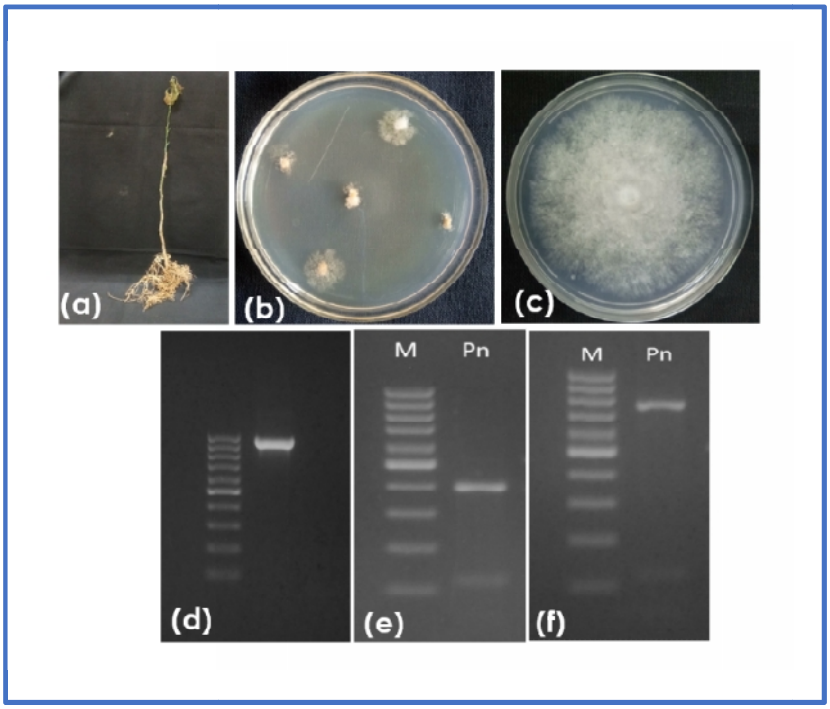

Fig. 1: (a) Symptoms of root rot of Citrus; (b) Isolation of *P. nicotianae* on PARPH medium; (c) Pure culture of *P. nicotianae* on CMA medium; (d) ~ 900 bp amplicon generated by ITS 6 and ITS 4 primer pair (e) 400 bp, and 120 bpbands on restriction digestion by *Msp*I; (f) 745,117,52bands on restriction digestion by *Alu*I.

In-vitro* evaluation of different fungicides against *P. Nicotianae. The present investigation was undertaken to evaluate the efficacy of different fungicides to restrict the mycelial growth of *P. nicotianae.* The results of *in-vitro* screening of fungicides at different concentrations are given in Table 2 (Fig. 2, 3 & 4). Complete mycelial inhibition of *P. nicotianae* was observed by Metalaxyl 4% + Mancozeb 64%, Fluopicolide (5.53% w/w) + Propamocarb hydrochloride (55.3% w/w), Metiram 44% + Dimethomorph 9%, Fluopicolide 39.5% and Mandipropamid 23.4%.

At 250 ppm, Fosetyl Al recorded 89.23 percent mycelial inhibition followed by Phosphorous acid (86.54%), Chlorothalonil 50% + Metalaxyl 3.75% SC (76.54%) and Cyazofamide (33.85%). The percent inhibition increased with the increase in concentrations of fungicides. Fosetyl Al and Phosphorous acid recorded mycelial inhibition of 92.31 and 100 percent at 500 and 1000 respectively. The mycelial inhibition by Azoxystrobin 20% + Difenoconazole 12.5% SC was recorded as 61.15, 83.08 and 88.08 per cent at 250, 500 and 1000 ppm respectively. The minimum mycelial inhibition was observed in Cyazofamide (43.85% at 500 ppm and 62.31% at 1000 ppm).

Table 2: Percent inhibition of *P. nicotianae* mycelium at different concentrations of fungicides.

Sr. No.	Fungicides	Chemical name	Mean percent inhibition		
			250 ppm	500 ppm	1000 ppm
Systemic Fungicides					
1.	Ridomil Gold	Metalaxyl 4% + Mancozeb 64%	100 (10.05)^{f}	100 (10.05)^{e}	100 (10.05)^{c}
2.	Alliette	Fosetyl Al	89.23 (9.499)^{e}	92.31 (9.66)^{d}	100 (10.05)^{c}
3.	Amistar Top	Azoxystrobin 20% + Difenoconazole 12.5% SC	61.15 (7.883)^{b}	83.08 (9.169)^{b}	88.08 (9.438)^{b}
4.	Ranman	Cyazofamide	33.85 (5.9)^{a}	43.85 (6.696)^{a}	62.31 (7.956)^{a}
5.	Prophyt	Phosphorous Acid	86.54 (9.356)^{d}	92.31 (9.66)^{d}	100 (10.05)^{c}
6.	Profiler	Fluopicolide 39.5%	100 (10.05)^{f}	100 (10.05)^{e}	100 (10.05)^{c}
7.	Acrobat	Metiram 44% + Dimethomorph 9%	100 (10.05)^{f}	100 (10.05)^{e}	100 (10.05)^{c}
Contact Fungicides					
8.	Revus	Mandipropamid 23.4%	100 (10.05)^{f}	100 (10.05)^{e}	100 (10.05)^{c}
Contact+systemic fungicides					
9.	Foliogold	Chlorothalonil 50% + Metalaxyl 3.75% SC	76.54 (8.805)^{c}	90.38 (9.56)^{c}	100 (10.05)^{c}
10.	Infinito	Fluopicolide 62.5% + Propamocarb hydrochloride 625 EC	100 (10.05)^{f}	100 (10.05)^{e}	100(10.05)^{c}
CD (P=0.05)			**0.0159**	**0.088**	**0.084**
SE(m)			**0.054**	**0.03**	**0.028**

*Figures in parenthesies are Arcsine transformed values. Values are mean of three replicates. Values with different letters are significantly different at p<0.05 level.

The fungicides with contact and contact+systemic mode of action *viz.,* Mandipropamid 23.4%, Chlorothalonil 50% + Metalaxyl 3.75% SC and Fluopicolide (5.53% w/w) + Propamocarb hydrochloride (55.3% w/w)were also tested at 2000 and 3000 ppm (Table 3). Complete mycelial inhibition was exhibited by all three fungicides at both the concentrations.

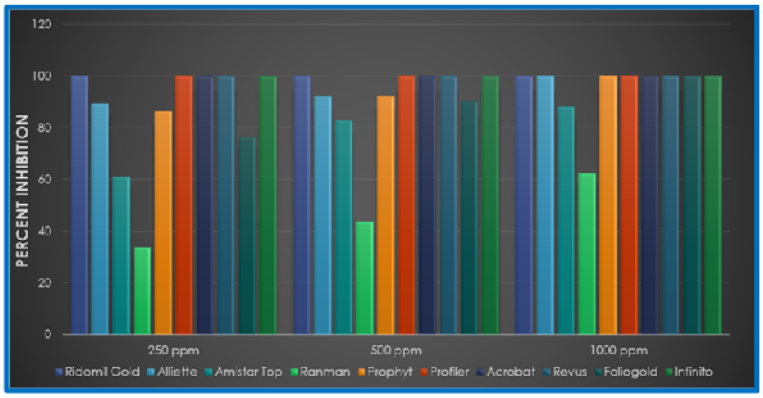

Fig. 2. Effect of different cocentrations (250, 500 and 1000 ppm) of ten different fungicides on mycelial growth of *P. nicotianae.*

Assessment of Minimum inhibitory concentrations (MIC's) of fungicidal dose for inhibition of *P. nicotianae* mycelium. Further, to study the MIC's of different fungicides, the concentrations of fungicides were reduced and increased accordingly. The concentrations of Azoxystrobin 20% + Difenoconazole 12.5% SC, Cyazofamide was increased (Table 3, Fig. 3 & 4). Complete inhibition of *P. nicotianae* mycelium by Azoxystrobin 20% + Difenoconazole 12.5% SC was recorded at 2000 ppm and that of Cyazofamide was recorded at 8000 ppm. From Table 4, it was evident that Fosetyl Al, Chlorothalonil 50% + Metalaxyl 3.75% SC, and Phosphorous acid completely inhibited growth of *P. nicotianae* at 1000 ppm.

Table 3: Mean values of percent inhibition of *P. nicotianae* mycelium at different concentrations of fungicides.

Sr. No.	Fungicides	Chemical name	Mean percent inhibition			
			2000 ppm	3000 ppm	5000 ppm	8000 ppm
1	Amistar Top	Azoxystrobin 20% + Difenoconazole 12.5% SC	90.39 (9.56)^{b}	92.31 (9.66)^{b}	100 (10.05)^{a}	NA
2	Ranman	Cyazofamide	73.08 (8.606)^{a}	75.71 (8.758)^{a}	80.77 (9.043)^{b}	100 (10.05)
3	Revus	Mandipropamid 23.4%	100 (10.05)^{c}	100 (10.05)^{c}	NA	NA
4	Foliogold	Chlorothalonil 50% + Metalaxyl 3.75% SC	100 (10.05)^{c}	100 (10.05)^{c}	NA	NA
5	Infinito	Flupicolide 62.5% + Promamocarb hydrochloride 625 EC	100 (10.05)^{c}	100 (10.05)^{c}	100 (10.05)^{a}	NA
CD (P=0.05)			**0.118**	**0.077**	**0.061**	**NA**
SE(m)			**0.037**	**0.024**	**0.015**	**NA**

*Figures in parenthesies are Arcsine transformed values. Values are mean of three replicates. Values with different letters are significantly different at p<0.05 level.

Similarly, to assess the MIC's of Metalaxyl 4% + Mancozeb 64%, Fluopicolide (5.53% w/w) + Propamocarb hydrochloride (55.3% w/w), Metiram 44% + Dimethomorph 9%, Fluopicolide 39.5% and Mandipropamid 23.4%, the concentrations were reduced (Table 4, Fig. 3 & 4). The result revealed that MIC of Metalaxyl 4% + Mancozeb 64%, Fluopicolide (5.53% w/w) + Propamocarb hydrochloride (55.3% w/w), Fluopicolide 39.5%, and Mandipropamid 23.4% required to completely inhibit the mycelial growth of *P. nicotianae* was 30 ppm while that of Metiram 44% + Dimethomorph 9% was 10 ppm.

Keeping in view the efficacy and cumulative performance, tested fungicides were grouped into three groups (group I, II and III). Group I consisted of highly effective fungicides *viz;* Metalaxyl 4% + Mancozeb 64%, Fluopicolide (5.53% w/w) + Propamocarb hydrochloride (55.3% w/w), Metiram 44% + Dimethomorph 9%, Fluopicolide 39.5% and Mandipropamid 23.4% that could completely inhibit the growth at 30 ppm and above. Second group included Fosetyl Al, Chlorothalonil 50% + Metalaxyl 3.75% SC and Phosphorous acid, as the fungus was moderately sensitive to these fungicides at 1000 ppm. The Group III included Azoxystrobin 20% + Difenoconazole 12.5% SC and Cyazofamide as they were least sensitive and could control the fungus at 2000 ppm and 8000 ppm respectively (Table 5).

Table 4: Percent inhibition of *P. nicotianae* mycelium at different concentrations of fungicides.

Sr. No.	Fungicides	Chemical name	Mean percent inhibition				
			5 ppm	10 ppm	30 ppm	50 ppm	100 ppm
1	Ridomil Gold	Metalaxyl 4% + Mancozeb 64%	NA	89.23 (9.499)[b]	100 (10.05)	100 (10.05)	100 (10.05)
2	Profiler	Fluopicolide 39.5%	NA	86.54 (9.356)[a]	100 (10.05)	100 (10.05)	100 (10.05)
3	Acrobat	Metiram 44% + Dimethomorph 9%	89.88 (9.533)	100 (10.05)[d]	100 (10.05)	100 (10.05)	100 (10.05)
4	Revus	Mandipropamid 23.4%	NA	90.39 (9.56)[c]	100 (10.05)	100 (10.05)	100 (10.05)
5	Infinito	Flupicolide 62.5% + Propamocarb hydrochloride 625 EC	NA	88.85 (9.479) [b]	100 (10.05)	100 (10.05)	100 (10.05)
CD (P=0.05)			NA	0.092	NS	NS	NS
SE(m)			NA	0.029	NS	NS	NS

*Figures in parenthesies are Arcsine transformed values. Values are mean of three replicates. Values with different letters are significantly different at p<0.05 level.

Table 5: Sensitivity of *P. nicotianae* mycelium to ten different fungicides.

Sr. No.	Fungicides	Chemical name	Sensitivity class	MIC's (ppm)	Sensitivity Group
1.	Acrobat	Metiram 44% + Dimethomorph 9%	HS	10	I
2.	Ridomil Gold	Metalaxyl 4% + Mancozeb 64%	HS	30	I
3.	Profiler	Fluopicolide 39.5%	HS	30	I
4.	Revus	Mandipropamid 23.4%	HS	30	I
5.	Infinito	Flupicolide 62.5% + Promamocarb hydrochloride 625 EC	HS	30	I
6.	Alliette	Fosetyl Al	MS	1000	II
7.	Foliogold	Chlorothalonil 50% + Metalaxyl 3.75% SC	MS	1000	II
8.	Prophyt	Phosphorous Acid	MS	1000	II
9.	Amistar Top	Azoxystrobin 20% + Difenoconazole 12.5% SC	LS	2000	III
10.	Ranman	Cyazofamide	LS	8000	III

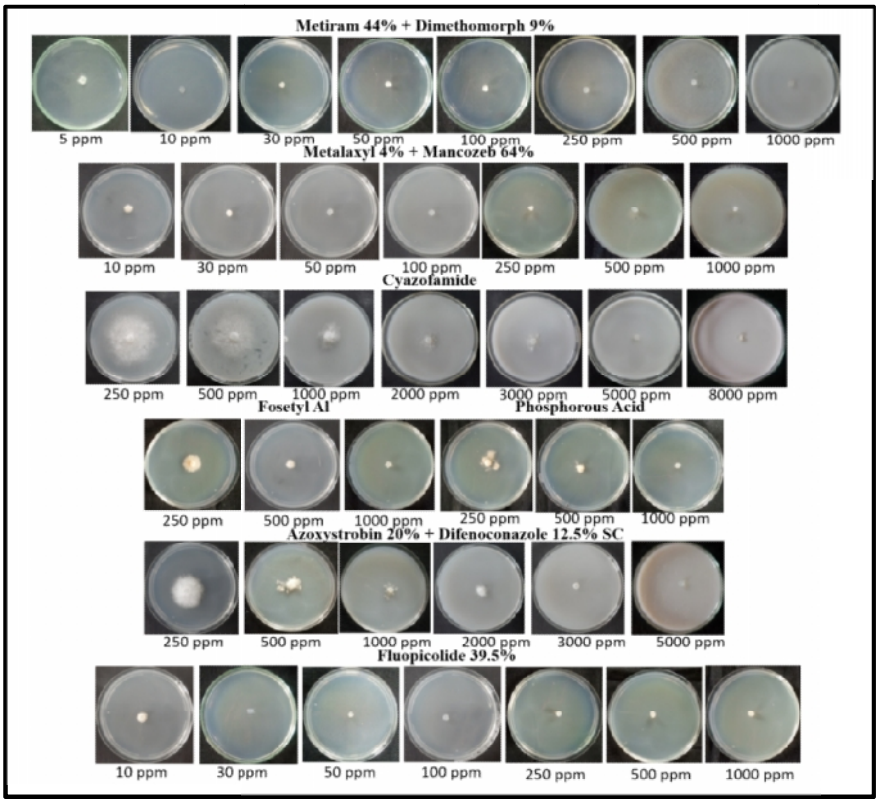

Fig. 3. Effect of different concentrations of systemic fungicides on mycelial growth of *P. nicotianae.*

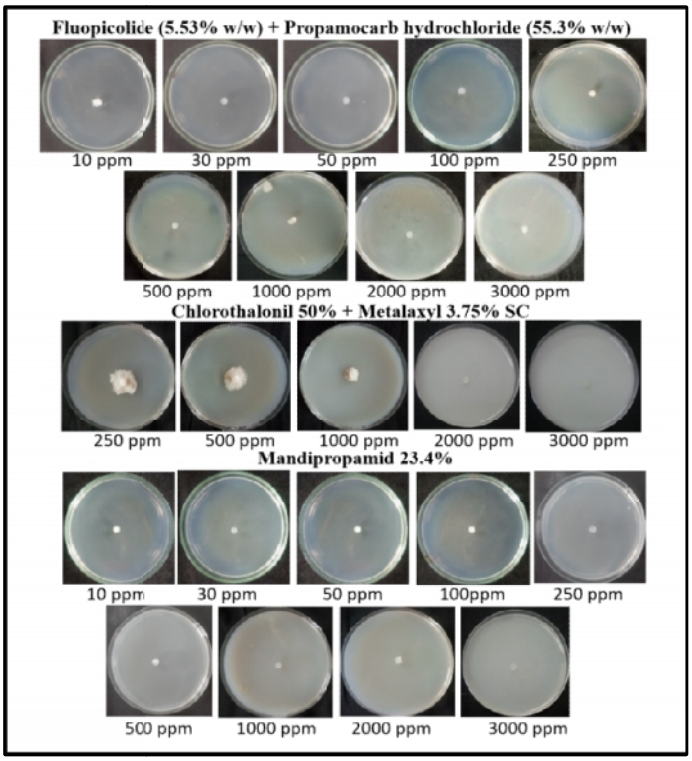

Fig. 4. Effect of different concentrations of contact and contact+systemic fungicides on mycelial growth of *P. nicotianae*.

DISCUSSION

Phytophthora nicotianae is the most devastating oomycetes of Citrus and hence, effective management strategies are needed to control the economical losses caused by this pathogen. The result obtained in the present study revealed that Metalaxyl 4% + Mancozeb 64, Fluopicolide (5.53% w/w) + Propamocarb hydrochloride (55.3% w/w), Metiram 44% + Dimethomorph 9%, fluopicolide 39.5% and mandipropamid 23.4%s strongly inhibited mycelial growth of *P. nicotianae*. Metalaxyl-m had been used since long in managing *P. nicotianae* in many crops (Farih *et al.,* 1981, Gonzalez *et al.,* 2017; Chi *et al.,* 2020). *In-vitro* inhibition mycelial growth of *Phytophthora* species by metalaxyl-m and mancozeb (ridomil) have been reported by Rolando *et al*. (2017); Turkolmez and Dervis (2017) and in pot trials (Reglinski *et al.,* 2009).

Though metalaxyl-m is highly effective in controlling the mycelial growth of *P. nicotianae,* many workers have reported the development of metalaxyl resistant strains (Davidse *et al.* 1981; Shew, 1985; Ferrin and Kabashima 1991; Gisi and Cohen 1996; Hwang and Benson 2005; Stack and Millar 1985; Parra and Ristaino 2001; Jeffers *et al*., 2004; Taylor *et al*., 2006; Hu *et al.,* 2008). Alternate use of fungicides with different mode of action and with low risk is important in reducing the risk of development of fungicide resistant strains.Therefore, the efficacy of fungicides with different mode action have been evaluated in present study.

Peerzada *et al*. (2020) reported that dimethomorph exhibited minimum mycelial growth of *P. infestans* while Yan *et al*., (2006); Matheran and Porchas (2000) reported the inhibitory effect on *P. nicotianae* and Jackson *et al.,* (2012); Siegenthaler *et al.* (2021) on *P. capsici.* The mode of action of dimethomorph is novel (*i.e.*,) it inhibits sterol synthesis that might have contributed to the high inhibitory effect. Since, it does not produce cross resistance to metalaxyl, are effective in low concentrations and poses systemic, curative and anti-sporulant spray which makes it effective even after infection sprays.

Fluopicolide plays a crucial role in breaking the stability of cytoskeleton, which works, by disorganization of cell structure of any pathogen and disrupting the formation of spectrin (Jiang *et al.,* 2015). The inhibitory effect of fluopicolide was also studied by many workers on mycelial growth of *P. capsici* (Jackson *et al.,* 2010), while on *P. nicotianae* (Qu *et al.,* 2013), *P. cinnamon* (Belisle *et al*., 2019) and other species by Cerkauskas *et al.* (2015); Foster and Hausbeck (2010); Jiang *et al.* (2015); Meyer and Hausbeck (2013); Shin *et al.* (2010). However, the inhibitory effect of propamocarb hydrochloride + fluopicolide was higher than the effect of fungicides when used alone (Ren *et al.,* 2018) which confirmed the findings of the present study. Propamocarb HCl interferes with the biosynthesis of membrane of oomycetes (Papavizas *et al.,* 1978). Combining the fungicides with different mode of action helps in reducing the resistant strains of pathogens.

Mandipropamid targets cellulose synthase (Kramer and Schirmer 2008) and the effectiveness of mandipropamid in controlling the root rot pathogen of citrus was reported by Gray *et al.* (2018); Hao *et al.* (2019), tobacco by Wang *et al.* (2013); Qu *et al.* (2013)

and vegetable crops (Cerkauskas *et al.*, 2015; Foster and Hausbeck 2010; Jiang *et al.* 2015; Meyer and Hausbeck 2013). Similarly, Belisle *et al*. (2019), reported the inhibitory effect of mandipropamid on *P. cinnamon* causing root rot of avocado.

Though the inhibitory concentrations of phosphorous acid, fosetyl Al, chlorothalonil and azoxystrobin was more, but they had significant effect on mycelial growth of *P. nicotianae.* Gonzalez *et al.* (2017) reported use of potassium phosphonate for controlling *P. cinnamon* in many forest, plantation, and horticultural tree species while Ramallo *et al.* (2019) reported 40 to 60 per cent reduction in incidence of brown rot of Citrus caused by *P. citrophthora*. Sandler *et al.* (1989); Sonoda *et al.* (1990); Gonzalez *et al.* (2017) reported the inhibitory effect of fosetyl Al against *Phytophthora* species. In present investigation, the inhibitory effect of fosetyl Al (Alliette) and phosphorous acid (Prophyt) were similar which were confirmed by the finding of Agosteo *et al.* (2010) who reported similar effects of phosphorous acid derivative (IR8465) and fosetyl Al. The greater inhibitory activity of dimethomorph, fosetyl Al and metalaxyl than that of azoxystrobin was confirmed by the Matheran and Porchas (2000). The inhibitory activity of azoxystrobin at 250, 500 and 1000 ppm was reported by Thomas and Naik (2017) and confirmed the findings of present study. The findings of Peerzada (2020) confirmed the least control of mycelial growth by chlorothalonil.

CONCLUSION AND FUTURE SCOPE

The use of fungicides in the laboratory and field depends on their *in-vitro* efficacy at minimal and economically acceptable dosages and their efficient and rapid transport to the infection site. Indiscriminate or inappropriate use can encourage the development of resistance in fungi. The high level of efficacy of five fungicides in our study (Metalaxyl, Fluopicolide, Mandipropamid, Propamocarb and dimethomorph), might help in alternating the use of fungicidal treatments to prevent development of fungicide resistance. Evaluation of MICs of ten fungicides helped to standardize the doses of fungicides against *P. nicotianae* along with traditionally used Ridomil gold in the present study. This study will be much helpful in future to devise fungicidal application schedule for commercial orchards.

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Conflict of Interest. None.

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