Agric Res J **60** (1) : 66-73, *February* 2023
DOI No. 10.5958/2395-146X.2023.00011.X

MANAGEMENT OF *Phytophthora nicotianae,* THE INCITANT OF CITRUS FOOT ROT, WITH NEW GENERATION FUNGICIDES

Yesmin Kaur*[1], Anita Arora[2] and S K Thind[1]

Department of Plant Pathology[1] and Department of Fruit Science[2], Punjab Agricultural University, Ludhiana-141004, Punjab

ABSTRACT

Foot rot caused by *Phytophthora nicotianae* is a serious disease in citrus nurseries and orchards. For its management, bio-efficacy of total nine fungicides (five systemic and combination, two systemic and two contact fungicides) was evaluated *in vitro* and under pot house conditions against citrus foot rot at Punjab Agricultural University, Ludhiana. Fluopicolide 4.44%+propamocarb hydrochloride 66.67%WG and metalaxyl 4%+mancozeb 64%WP effectively controlled the mycelial growth with ED50 value of 0.8µg/ml followed by cymoxanil 8%+mancozeb 64%WP (ED50 value 18.0µg/ml), while chlorothalonil 75%WP was the least effective in inhibiting mycelial growth of the pathogen (ED50 value 328.0µg/ml). Under field conditions, metalaxyl 4%+mancozeb 64%WP was the most effective in controlling the pathogen population and increasing shoot and root length, seedling canopy, leaf area, number of rootlets, root volume and density of Rough lemon seedlings as compared to fluopicolide 4.44%+propamocarb hydrochloride 66.67%WG and cymoxanil 8%+mancozeb 64%WP. Seedlings treated with fosetyl aluminium 80%WP had significantly lesser shoot and root growth. All the treatments had root rot rating of 1 as compared to 4 in untreated check.

Keywords: Citrus foot rot, *Phytophthora nicotianae,* Fungicide screening, Management

Citrus foot rot caused by *Phytophthora nicotianae* is a major threat to citrus cultivation in nurseries and orchards (Thind and Sharma, 1996; Das, 2009). The disease causes 10 to 30 per cent losses in citrus plantations around the world (Timmer *et al.*, 2000). It causes moderate to heavy destruction of Kinnow plantations and is responsible for the orchard decline in Punjab (Thind *et al.,* 2004; Kaur *et al*., 2011). The disease can be managed by using disease free planting material (Naqvi, 2004), application of fungicides (Dhakad *et al.,* 2016), soil amendments (Singh *et al*., 2015) and biocontrol by bioagents (Singh *et al.,* 2011) as trunk paint (Thind *et al.,* 1999), soil drench and sprays (Thind *et al.,* 2004). The repeated use of fungicides leads to the development of resistant strains of *Phytophthora.* Keeping in view, the present investigation was undertaken to test and compare the efficacy of different fungicides against citrus *Phytophthora* for the safe management of the disease.

MATERIALS AND METHODS

The *in vitro* efficacy of fungicides was conducted at Laboratory of Plant Pathology, Department of Fruit Science and *in vivo* experiment was conducted at Pot House, Department of Plant Pathology, PAU, Ludhiana.

Fungal isolation

Rhizosphere soil of infected Kinnow tree from College orchard, Department of Fruit Science was collected and twenty grams of sample was added in 80 ml water to make a total volume of 100 ml. One ml of the soil suspension (in soil-water ratio of 1:4) was poured into each Petri plate containing selective PARPH-CMA medium (containing Pimaricin @ 0.01gm/lt, Ampicillin @ 0.25 gm/lt, Rifampicin @ 0.012 gm/lt, Pentachloronitrobenzene @ 0.01 gm/lt and Hymexazol @ 0.08 gm/lt in Corn meal agar @ 17 gm/lt) under aseptic conditions and incubated in a BOD incubator at 25±1°C for 24 hours. Fungal colonies were sub-cultured in separate Petri plates containing corn meal agar medium without antibiotics to obtain the pure culture of *Phytophthora nicotianae.* The pathogen was identified on the basis of morphological characteristics of sporangia as described by Waterhouse (1963) and through molecular identification using species specific primers (as described by Ippolito *et al.* 2002).

Mass culture of pathogen

A pure culture was then used for mass culturing of *P. nicotianae* on sorghum seeds. Sorghum seeds were washed in tap water and soaked overnight. Fifty ml of sterile distilled water was added to 50 gm of sorghum seeds and autoclaved three times. After autoclaving, seeds were inoculated with 5 mycelial discs (5 mm) of the fresh culture of *P. nicotianae* under aseptic

*Corresponding author : yesminkaur@pau.edu
Date of receipt: 04.05.2022, Date of acceptance: 28.02.2023

conditions and incubated at 25±1°C in BOD incubator for 15 days. The sorghum seeds colonized by *P. nicotianae* were then used for inoculating one year old seedlings of rough lemon to induce pathogenecity.

In vitro efficacy of fungicides

Nine fungicides namely, metalaxyl 4% + mancozeb 64% WP, cymoxanil 8% + mancozeb 64% WP, fosetyl aluminium 80% WP, fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG, fenamidone 10% + mancozeb 50% WG, mandipropamid 250 SC, famoxadone 16.6% + cymoxanil 22.1% SC, chlorothalonil 75% WP and propineb 70% WP were tested *in vitro* to compare their efficacy against *Phytophthora nicotianae* by Poison food assay (Nene and Thapliyal, 1993). A series of concentrations of 1, 10, 50, 100, 200 and 500 µg/ml were prepared by serial dilution in sterile distilled water. Calculated amount of each fungicide was added to sterile corn meal agar medium and the fungicide amended medium (20 ml) was poured into Petri plates under aseptic conditions. Petri plates containing medium without any amended fungicide served as untreated control. Five mm mycelial disc of 7-days old culture of *P. nicotianae* was aseptically transferred to the center of each Petri plate. Five replications of each concentration of fungicide treatment were kept and the Petri plates were incubated at 25±1°C in BOD incubator. Radial colony growth was measured in all the treatments when the control plates were full with mycelial growth of the fungus and growth inhibition in each concentration of fungicide was calculated by the following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{Colony growth in control} - \text{Colony growth in treatment}}{\text{Colony growth in control}} \times 100$$

In vivo efficacy of fungicides

One year old disease free Rough lemon seedlings were obtained from nursery grown in Screen house, College Orchard, Department of Fruit Science in 2017. The seedlings were transplanted in pots containing sterilized soil with one seedling per pot and pots were kept in Pot house of the Department of Plant Pathology, PAU, Ludhiana. Each pot was inoculated with 60 ml of sporangial suspension having 20 sporangia/ml strength by making 6 cm deep and 2 cm diameter holes in the pot. The holes were closed with the same soil and pots were watered at weekly intervals to maintain the moisture. Fungicides found effective under *in vitro* conditions i.e. metalaxyl 4% + mancozeb 64% WP, cymoxanil 8% + mancozeb 64% WP and fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG were applied as soil drench along with spraying recommended fungicide, fosetyl aluminium 80% WP, after 15 days of inoculations. Each treatment was replicated five times with one seedling per replication in each pot. The experiment was repeated twice in 2018 also.

Population propagule recovery was recorded from the rhizosphere by soil plating method after 30 days of fungicide application in 2017 and 2018. Twenty grams of soil sample was added to 80 ml sterile distilled water to a make total volume of 100 ml and one ml of the soil suspension (in soil-water ratio of 1:4) was poured into each Petri plate containing selective PARPH-CMA medium under aseptic conditions. The plates were incubated in BOD incubator at 25±1°C for 24 hours to count the number of *Phytophthora* colonies. The data was analyzed in a completely randomized design with DMRT using SAS software (version 9.2, SAS Institute Inc., Cary, NC, USA).

The seedlings were carefully uprooted by keeping their roots intact and seedling growth parameters *viz.* shoot and root length, number of leaves and branches, number of rootlets and stem girth were measured. The canopy was calculated by the formula of Coder (2000):

Canopy volume (cc) = 0.5236 × height × spread (N-S) × spread (E-W)

Chlorophyll content of the leaves was estimated by the method of Lichtenthaler (1987) using formula:

$$\text{Total chlorophyll (mg/g tissue)} = [20.0\ (OD)\ 645 + 8.02\ (OD)\ 663] \times \frac{v}{1000 \times w}$$

where OD is optical density of chlorophyll extract; v is final volume of 80 per cent acetone chlorophyll extract; and w is initial weight of leaf sample.

The root volume of the seedlings was recorded by water-displacement method of Harrington *et al.* (1994). Root density was calculated by the formula:

$$\text{Root density (mg/cc soil)} = \frac{\text{Dry weight of roots}}{\text{Total root volume}}$$

The observation on the root rot decay by *Phytophthora* was recorded as per scale (1-5) given by Grimm and Hutchinson (1973):

1 = No visible symptoms

2 = A few roots with symptoms (1-25%)

3 = Majority of roots with symptoms (26-50%)

4 = All roots infected and cortex sloughed (51-75%)

5 = Majority of roots dead or missing (76-100%)

Statistical analysis

Mycelial growth inhibition data were analyzed statistically in a completely randomized design using SAS software (version 9.2, SAS Institute Inc., Cary, NC, USA). ED_{50} values were calculated by plotting per cent mycelial growth inhibition against different concentrations of each test fungicide. The pot-house seedlings growth data in different treatments were

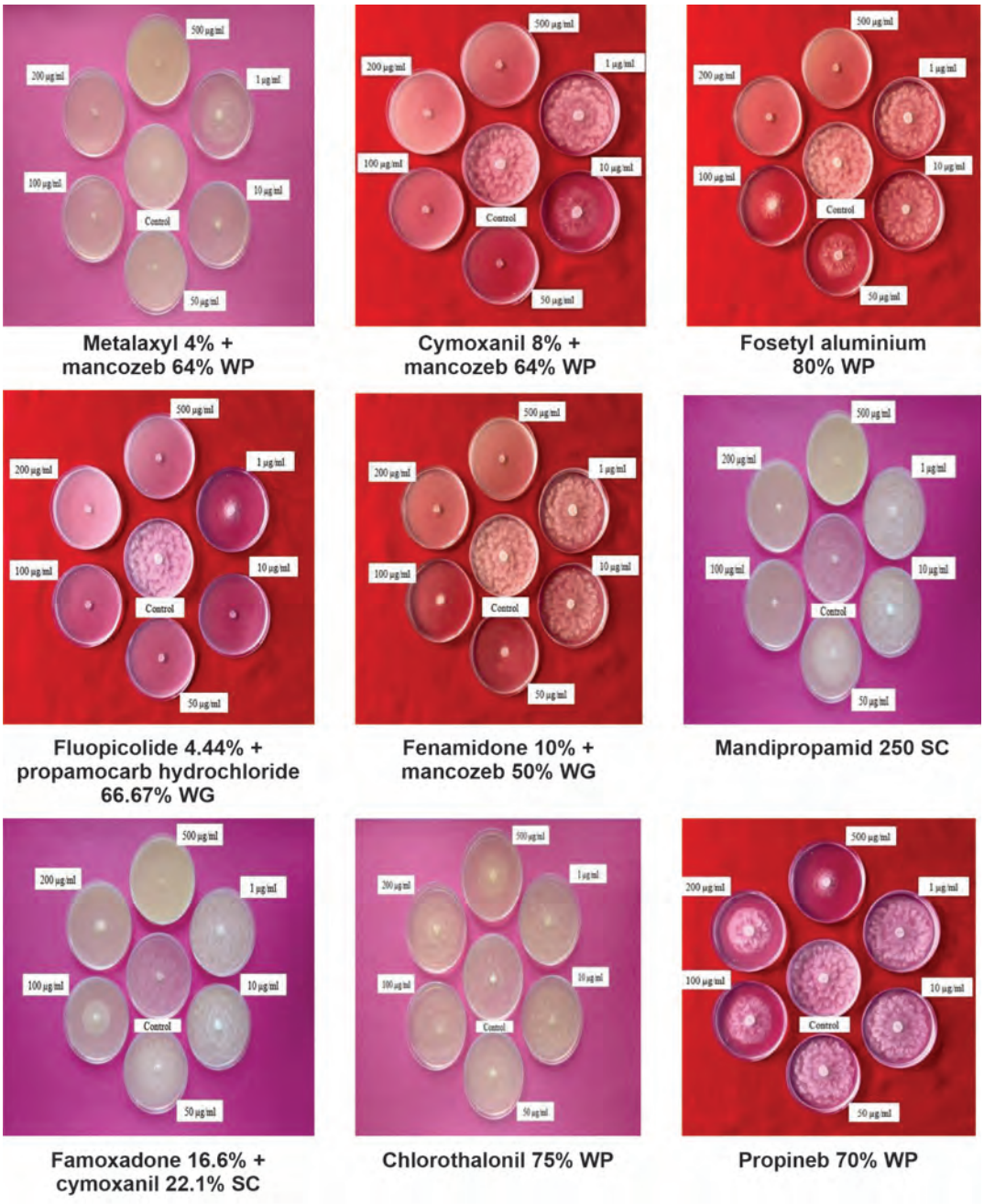

Fig. 1. Growth inhibition of *Phytophthora nicotianae* by different fungicides

analyzed in completely randomized block design and box plots were created. Percent increase in growth of fungicide treated plants over the untreated check plants was calculated.

RESULTS AND DISCUSSION

Fungal isolation

Suspension of soil taken from rhizosphere of infected Kinnow trees from College orchard, Department of Fruit Science was used for *P. nicotianae* isolation. Individual white mycelial colonies of the pathogen appeared on the Petri plates after seven days of incubation in BOD incubators at 25±1 °C. *P. nicotianae* was identified on the basis of spherical to ovoid sporangia with prominent papilla and through polymerase chain reaction using species specific primers. Mass culturing of pure culture was done on sorghum seeds and rough lemon seedlings were used for pathogenicity. Symptoms like vertical stem cracks, gummosis and rotting of secondary roots were visible after 30 days of inoculations.

In vitro efficacy of fungicides

All the fungicides were effective in controlling mycelial growth of the pathogen at varying concentrations (Fig. 1). Significantly maximum mycelial growth inhibition was recorded by fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG followed by metalaxyl 4% + mancozeb 64% WP with mean growth inhibition of 94.0 and 93.1 per cent, respectively (Table 1). While minimum mean growth inhibition was recorded in chlorothalonil 75% WP with mean growth inhibition of 30.7 per cent. A cent per cent growth inhibition was recorded by fluopicolide 4.44% + propamocarb hydrochloride 66.67% WP and metalaxyl 4% + mancozeb 64% WP at 10, 50, 100, 200 and 500 µg/ml concentrations. Cymoxanil 8% + mancozeb 64% WP and fenamidone 10% + mancozeb 50% WG had 36.7and 15.1 per cent growth inhibition at 10 µg/ml while at 50 µg/ml, they achieved cent per cent inhibition. Fosetyl aluminium 80% WP and mandipropamid 250 SC had 58.7 and 51.7 per cent inhibition at 100 µg/ml and exhibited cent per cent inhibition at 200 µg/ml. While famoxadone 16.6% + cymoxanil 22.1% SC had 98.5 per cent inhibition at 200 µg/ml and cent per cent inhibition was recorded at 500 µg/ml. Chlorothalonil 75% WP and propineb 70% WP were the least effective against *Phytophthora* growth. They did not show complete growth inhibition at any concentration. Growth inhibition of 58.7 and 80.8 per cent was recorded at 500 µg/ml of chlorothalonil 75% WP and propineb 70% WP, respectively.

The findings are in agreement with Thind *et al.* (2004) who recorded a cent per cent growth inhibition at the lowest tested dose of 50 µg/ml in pure metalaxyl WP and metalaxyl 4% + mancozeb 64% WP while fosetyl aluminium 80% WP produced 50.58 and 100.00 per cent growth inhibition at 1000 and 2500 µg/ml, respectively. Similarly, Kaur *et al.* (2011) reported that metalaxyl 4% + mancozeb 64% WP and cymoxanil 8% + mancozeb 64% WP were effective in controlling cent per cent mycelial growth at 25 and 50 µg/ml, respectively, while fosetyl aluminium 80% WP had not controlled the mycelial growth completely. They also reported ED_{50} values of metalaxyl 4% + mancozeb 64% WP, cymoxanil 8% + mancozeb 64% WP and fosetyl aluminium 80% WP as <25, 25 and 97 µg/ml, respectively. Similarly, Dhakad *et al.* (2016) achieved cent per cent growth inhibition at 10 µg/ml of metalaxyl 4% + mancozeb 64% WP while fluopicolide and cymoxanil 8% + mancozeb 64% WP had at 50 µg/ml. On the contrary, Jagtap *et al.* (2012) recorded highest per cent growth inhibition by cymoxanil 8% + mancozeb

Table 1. *In vitro* efficacy of different fungicides to *Phytophthora nicotianae*

Fungicides	Growth inhibition (%) 1µg/ml	10µg/ml	50µg/ml	100µg/ml	200µg/ml	500µg/ml	Mean Growth inhibition	ED_{50} (µg/ml)
Metalaxyl 4% + mancozeb 64% WP	58.4^{d} (49.8)	100.0^{a} (88.3)	100.0^{a} (88.3)	100.0^{a} (88.3)	100.0^{a} (88.3)	100.0^{a} (88.3)	93.1 (80.4)	0.8
Cymoxanil 8% + mancozeb 64% WP	7.5^{l,m,n} (15.9)	36.7^{g} (37.2)	100.0^{a} (88.3)	100.0^{a} (88.3)	100.0^{a} (88.3)	100.0^{a} (88.3)	74.0 (61.8)	18.0
Fosetyl aluminium 80% WP	6.7^{l,m,n,o} (14.9)	15.3^{k} (22.9)	28.8^{h} (32.4)	58.7^{d} (49.9)	100.0^{a} (88.3)	100.0^{a} (88.3)	51.6 (45.9)	86.0
Fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG	63.9^{c} (53.1)	100.0^{a} (88.3)	100.0^{a} (88.3)	100.0^{a} (88.3)	100.0^{a} (88.3)	100.0^{a} (88.3)	94.0 (81.0)	0.8
Fenamidone 10% + mancozeb 50% WG	6.3^{m,n,o} (14.5)	15.1^{k} (22.8)	100.0^{a} (88.3)	100.0^{a} (88.3)	100.0^{a} (88.3)	100.0^{a} (88.3)	70.2 (59.9)	26.0
Mandipropamid 250 SC	4.7^{n,o} (12.5)	18.3^{j,k} (25.3)	22.3^{j,i} (28.2)	51.7^{e} (45.9)	100.0^{a} (88.3)	100.0^{a} (88.3)	49.5 (44.8)	96.0
Famoxadone 16.6% + cymoxanil 22.1% SC	6.3^{m,n,o} (14.5)	10.2^{l,m} (18.6)	20.9^{j} (27.2)	64.8^{c} (53.6)	98.5^{a} (83.1)	100.0^{a} (88.3)	50.1 (45.4)	83.0
Chlorothalonil 75% WP	3.4^{o,p} (10.6)	10.3^{l,m} (18.7)	25.6^{h,i} (30.4)	42.3^{f} (40.6)	44.0^{f} (41.5)	58.7^{d} (49.9)	30.7 (31.2)	328.0
Propineb 70% WP	0.0^{p} (1.7)	10.5^{l} (18.9)	27.6^{h} (31.7)	43.6^{f} (41.3)	48.8^{e} (44.3)	80.8^{b} (64.0)	35.2 (36.7)	214.0

Values in parenthesis are arc sine transformed values
LSD (p=0.05) Fungicides=0.79
Concentrations=0.64
Fungicides×Concentrations=1.53

64% WP followed by metalaxyl 4% + mancozeb 64% WP.

The ED_{50} values were less in fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG (0.8 µg/ml) and metalaxyl 4% + mancozeb 64% WP (0.8 µg/ml) followed by cymoxanil 8% + mancozeb 64% WP (18.0 µg/ml), fenamidone 10% + mancozeb 50% WG (26.0 µg/ml), famoxadone 16.6% + cymoxanil 22.1% SC (83.0 µg/ml), mandipropamid 250 SC (96.0 µg/ml), fosetyl aluminium 80% WP (86.0 µg/ml) and were higher in propineb 70% WP (214.0 µg/ml) and chlorothalonil 75% WP (328.0 µg/ml).

In vivo efficacy of fungicides

Phytophthora propagule count was recovered from the rhizosphere of seedlings treated with metalaxyl 4% + mancozeb 64% WP, fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG, cymoxanil 8% + mancozeb 64% WP and untreated control. The propagule count recovered from the rhizosphere of untreated seedlings was significantly maximum (85.60 and 79.93 cfu/ cc soil) in both consecutive years followed by seedlings sprayed with fosetyl aluminium 80% WP (18.1 and 16.4 cfu/ cc soil) (Table 2). There was no significant difference observed in population recovery from treatments of fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG (4.4 and 3.8 cfu/ cc soil) and metalaxyl 4% + mancozeb 64% WP (4.4 and 4.0 cfu/ cc soil) while cymoxanil 8% + mancozeb 64% WP drenched seedlings had significantly higher population levels (7.3 and 5.4 cfu/ cc soil) in both testing years. The present findings are in similarity to Farih *et al.* (1981) who recovered the *Phytophthora* population after the application of fosetyl aluminium while no population was recovered with metalaxyl application. Similarly, Thind *et al.* (2004) recorded more propagules count in fosetyl aluminium 80% WP (12.5 cfu/cc soil) than in metalaxyl 4% + mancozeb 68% WP (7.5 cfu/cc soil).

Seedlings drenched with fungicides produced more vigorous shoot growth as compared to unsprayed check plants of rough lemon (*C. jambhiri*). Significantly, maximum shoot length (57.20 and 61.60 cm) and number of leaves (52.80 and 64.20) were recorded in seedlings drenched with metalaxyl 4% + mancozeb 64% WP as compared to untreated control (40.40 and 41.10 cm and 37.60 and 44.60, respectively) in both testing years (Fig. 2). Significantly more canopy was recorded in seedlings drenched with metalaxyl 4% + mancozeb 64% WP (0.018 and 0.025, respectively in 2017 and 2018), fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG (0.025 and 0.017, respectively in 2017 and 2018) and cymoxanil 8% + mancozeb 64% WP (0.026 and 0.016, respectively in 2017 and 2018) than the seedlings sprayed with fosetyl aluminium 80% WP (0.014 and 0.021, respectively in 2017 and 2018) and untreated control (0.012 and 0.005, respectively in 2017 and 2018). Total chlorophyll content was significantly more in fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG (1.83 and 1.68 mg/g tissue) and less in cymoxanil 8% + mancozeb 64% WP (1.38 and 1.41mg/g tissue, respectively in 2017 and 2018). Maximum root length was recorded in seedlings treated with metalaxyl 4% + mancozeb 64% WP (35.7 and 26.7 cm) and was significantly higher than the seedlings treated with fosetyl aluminium 80% WP (26.2 and 29.5 cm, respectively in 2017 and 2018) (Fig. 2). Root length of seedlings treated with fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG (29.8 and 28.4 cm) and cymoxanil 8% + mancozeb 64% WP (25.6 and 34.0 cm) was at par with each other. Significantly higher root density was recorded in the seedlings treated with cymoxanil 8% + mancozeb 64% WP (448.57 and 472.53 mg/cc soil, respectively in 2017 and 2018) followed by metalaxyl 4% + mancozeb 64% WP (319.32 and 305.39 mg/cc soil), fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG (352.77 and 319.32 mg/cc soil) and fosetyl aluminium 80% WP (305.39 and 310.59 mg/cc soil, respectively in 2017 and 2018).

The shoot length and the number of leaves of the seedlings drenched with fluopicolide 4.44% +

Table 2. Recovery of *Phytophthora nicotianae* from rhizosphere of Rough lemon seedlings treated with different fungicides

Treatments	Population propagules per cc soil*		Detection by Immunostrips
	Ist year	IInd year	
Metalaxyl 4% + mancozeb 64% WP	4.40 [a]	4.00 [a]	-
Fosetyl aluminium 80% WP	18.10 [c]	16.40 [c]	-
Cymoxanil 8% + mancozeb 64% WP	7.30 [b]	5.40 [b]	-
Fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG	4.40 [a]	3.80 [a]	+
Control	85.60 [d]	79.93 [d]	+
LSD (p=0.05)	1.33	0.59	

*Mean of two years

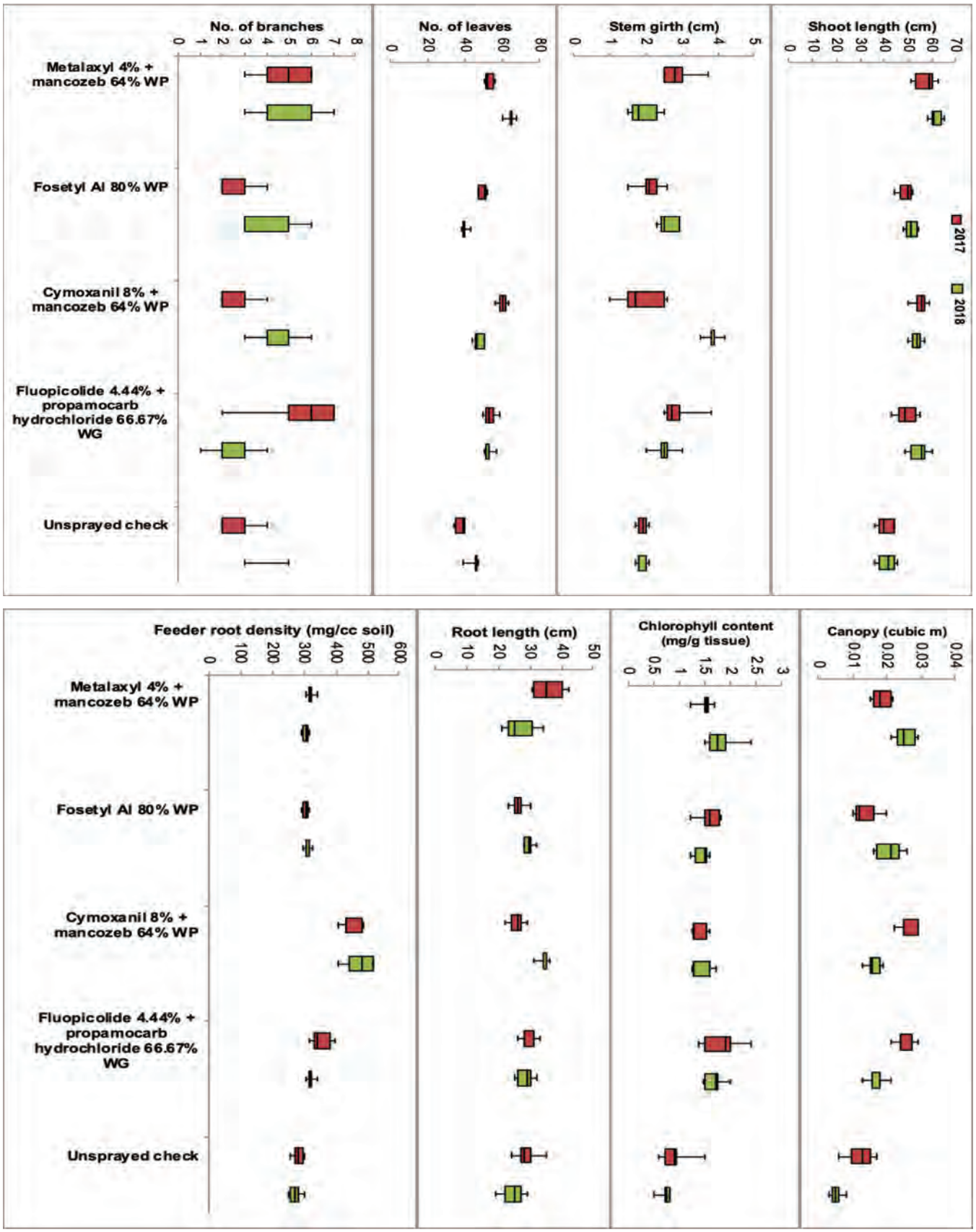

Fig. 2. Effect of different fungicides on shoot and root parameters of Rough lemon seedlings

Table 3. Per cent increase in shoot growth of Rough lemon seedlings in different fungicide treatments over the unsprayed check

Fungicide treatments	Concentration (%)	Increase over control (%)						
		Shoot length	Stem girth	Canopy	Branches	Leaves	Leaf area	Chlorophyll
Metalaxyl 4% + mancozeb 64% WP	0.25	49.80	37.93	153.38	50.00	43.09	52.55	96.41
Fosetyl Al 80% WP	0.25	24.70	18.97	89.43	10.00	8.94	41.23	76.30
Cymoxanil 8% + mancozeb 64% WP	0.25	29.96	37.93	140.36	10.00	30.08	47.10	67.32
Fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG	0.25	32.79	39.66	136.25	20.00	29.27	50.47	113.80

Table 4. Per cent increase in root growth of Rough lemon seedlings in different fungicide treatments over the unsprayed check

Fungicide treatments	Concentration (%)	Increase over control (%)				
		Root length	Rootlets	Root volume	Root density	Root rot rating
Metalaxyl 4% + mancozeb 64% WP	0.25	17.61	16.07	90.00	12.67	1
Fosetyl Al 80% WP	0.25	5.03	9.82	40.00	11.08	1
Cymoxanil 8% + mancozeb 64% WP	0.25	12.58	18.75	45.00	66.19	1
Fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG	0.25	10.06	17.86	65.00	21.13	1

propamocarb hydrochloride 66.67% WG (32.79 and 29.27% increase over untreated check) and cymoxanil 8% + mancozeb 64% WP (29.96 and 30.08% increase over untreated check) was at par with each other (Table 3). The number of branches (10 to 50% increase) and stem girth (18.97 to 39.66% increase) of seedlings was at par with each other. The maximum number of rootlets were produced in seedlings treated with cymoxanil 8% + mancozeb 64% WP (18.75% increase) followed by fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG (17.86% increase) while the minimum was in seedlings treated with fosetyl aluminium 80% WP (9.82% increase over untreated check) (Table 4). Significantly maximum root volume was recorded in seedlings treated with metalaxyl 4% + mancozeb 64% WP (90% increase) followed by fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG (65% increase over untreated check). The root volume in seedlings treated with cymoxanil 8% + mancozeb 64% WP was at par with the seedlings treated with fosetyl aluminium 80% WP (45% and 40% increase over untreated check, respectively). The feeder root was intact and free from disease symptoms in all the fungicide treatments (root rot rating of 1) while feeder root damage was 4 in seedlings of the unsprayed check.

The studies are in accordance with Thind *et al.* (1999) who reported maximum recovery of chlorophyll (52.91%) and tree volume (32.26%) in plants treated with metalaxyl 4% + mancozeb 64% WP over untreated control. Sandler *et al.* (1986), Sawant *et al.* (1990) and Thind *et al.* (2004) reported the effectiveness of metalaxyl 4% + mancozeb 68% WP and fosetyl aluminium 80% WP in reducing trunk lesion size, increasing feeder root density and tree productivity in Etrog citron, Coorg mandarin and Kinnow. Similarly, Kaur *et al.* (2011) reported more seedling height, number of leaves, feeder root length and volume in metalaxyl 4% + mancozeb 64% WP and cymoxanil 8% + mancozeb 64% WP than in untreated control. Singh *et al.* (2015) also reported 54 per cent lesion recovery with the application of metalaxyl and fosetyl aluminium 80% WP. Likewise, Graham *et al.* (2017) recommended the application of metalaxyl 4% + mancozeb 68% WP and fosetyl aluminium 80% WP in Florida for the management of citrus *Phytophthora* disease.

The study shows that combination of fungicides, fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG and metalaxyl 4% + mancozeb 64% WP were effective in cent per cent growth inhibition at 10 µg/ml followed by cymoxanil 8% + mancozeb 64% WP and fenamidone 10% + mancozeb 50% WG which produced cent per cent inhibition at 50 µg/ml. Metalaxyl 4% + mancozeb 64% WP and fluopicolide 4.44% + propamocarb hydrochloride 66.67% WG were found more effective under *in vivo* conditions during both the years of testing when seedlings were drenched with 0.25 per cent fungicide solution.

Authors' contribution

Conceptualization of research work and designing of experiments(AA, SKT); Execution of field/lab experiments and data collection(YK); Data analysis and interpretation of results(YK, AA, SKT); Preparation of manuscript(YK, AA).

LITERATURE CITED

Coder K D 2000. Tree biomechanics series: crown shape factors and volumes. https://urbanforestrysouth.org/resources/library/citations/Citation.2005-12-07.0632/at_download/file

Das A K 2009. *Fungal diseases in citrus and its management: Integrated management in citrus*. Bulletin Pp. 53-57. National Research Center for Citrus, Nagpur.

Dhakad U K, Kaur S and Thind S K 2016. *In vitro* comparative efficacy of fungicides against *Phytophthora nicotianae* var. *parasitica* causing foot rot of citrus. *Plant Dis Res* **31:** 122-24.

Farih A, Menge J A, Tsao P H and Ohr H D 1981. Metalaxyl and efosite aluminum for control of *Phytophthora* gummosis and root rot on citrus. *Plant Dis* **65:** 654-57.

Graham J H, Dewdney M M and Johnson E G 2017. *Phytophthora* Foot Rot and Root Rot. In *Florida citrus production guide*. Pp.111-14. University of Florida.

Grimm G R and Hutchison D J 1973. A procedure for evaluating resistance of citrus seedlings to *Phytophthora parasitica*. *Pl Dis Rep* **57:** 669-72.

Harrington J T, Mexal J G and Fisher J T 1994. Volume displacement provides quick and accurate way to quantify new root production. *Tree pl Not* **45:** 121-24.

Ippolito A, Schena L and Nigro F 2002. Detection of *Phytophthora nicotianae* and *P citrophthora* in citrus roots and soils by nested PCR. *Eur J Plant Pathol* **108:** 855-68.

Jagtap G J, Dhavale M C and Dey U 2012. Evaluation of natural plant extracts, antagonists and fungicides in controlling root rot, collar rot, fruit (brown) rot and gummosis of citrus caused by *Phytophthora* spp. *in vitro. Sci J Microbiol* 1: 27-47.

Kaur A, Verma K S and Thind S K 2011. *In vitro* screening of fungicides against citrus foot rot (*Phytophthora parasitica*). *Plant Dis Res* 24: 90-92.

Lichtenthaler H K 1987. Chlorophyll and carotenoids: pigments of photosynthesis biomembranes. In Packer L and Dauce R (ed) *Methods in enzymology: plant cell membranes* **148:** 350-82, Academic Press.

Naqvi S A M H 2004. Diagnosis and management of certain important fungal diseases of citrus. In: Naqvi S A M H (ed). *Diseases of fruits and vegetables: diagnosis and management*. Pp. 247-90. Kluwer Academic Publishers, Springer Science.

Nene Y L and Thapliyal P N 1993. *Fungicides in plant disease control*. Pp.579. Oxford and IBH Publishing Co., New Delhi, India.

Sandler H A, Timmer L W and Graham J H 1986. Timing of application of metalaxyl and fosetyl-aluminium for the control of *Phytophthora* foot rot. *Proc Florida St Horte Soc* **99:** 57-59.

Sawant S D, Manjunath K L and Sawant I S 1990. Chemical control of stump rot and gummosis of Coorg mandarin in Kodagu. *Plant Dis Res* **5:** 191-93.

Singh N, Singh R, Thind S K and Singh P 2011. Sampling kinnow orchards in Punjab to estimate populations of antagonistic microflora and its screening against *Phytophthora parasitica* causing foot rot in citrus. *Plant Dis Res* **26:** 35-39.

Singh R, Dalal R P S and Bhatia S K 2015. Management of citrus foot rot/gummosis through integration of agronomic practices, bioagent and chemicals. *Indian J Plant Prot* **43:** 350-53.

Thind S K and Sharma J N 1996. Incidence and control of citrus gummosis in Kinnow manadarin. *Indian J Hort* **53:** 118-20.

Thind S K, Sharma J N and Josan J S 1999. Comparative efficacy of fungicides and their methods of application for the control of foot rot of citrus. *Plant Dis Res* 14: 155-60.

Thind S K, Thind T S and Arora J K 2004. Prevalence of citrus *Phytophthora* in Kinnow mandarin orchards and its fungicidal management. *Plant Dis Res* **19:** 31-35.

Timmer L W, Garnsey S M and Graham J M 2000. *Compendium of citrus diseases*. pp. 156-62. APS Press, St. Paul. Minnesota, USA.

Waterhouse G M 1963. Key to the Species of *Phytophthora* de Bary. *Mycological Papers.* pp. 1-22. Commonwealth Mycological Institute, Kew, Surrey, England.