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Eco-friendly Management of *Phytophthora* Root Rot and Gummosis in Mandarin

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Abstract: The oomycete *Phytophthora nicotianae* causes root rot, foot rot, crown rot, and gummosis in the mandarin crop throughout India. Most mandarin root stocks grown in country is susceptible, or at best moderately susceptible, and necessitates frequent fungicide applications to avoid heavy yield losses. Four years of field trials in three different locations (Akola, Ludhaina and Tinsukia) investigated the effect of different conventional and one non-conventional chemical integration with bio-agents on *Phytophthora* root rot and gummosis. The foliar spraying of potassium phosphonate (potassium salt of phosphonic acid) at 3 g/liter water was superior in terms of average reduction in lesion oozing (51.54%), minimum feeder root rot index (1.07), increase in canopy volume (7.93%), and higher fruit yield (17.84 t/ha). Potassium phosphonate is known to induce defense responses in mandarin as well as to have direct toxic effects on oomycetes, which in turn inhibits the development of root rot and gummosis. The use of potassium phosphonate in mandarin crops has been promoted as a feasible method as part of the integrated disease management strategy for *Phytophthora* root rot and gummosis management.

Keywords: Gummosis, Mandarin, Potassium phosphonate and Phytophthora

The mandarin (*Citrus reticulata* Blanco) is the most important commercial Citrus cultivar in India, with a total production of 13976MT (NHB 2019). Commercially, Nagpur mandarin is grown in the Vidarbha region of Maharashtra and adjoining areas of Madhya Pradesh; Khasi mandarin in the north eastern region; Kinnow mandarin in Punjab, Haryana, the western part of Rajasthan, Uttar Pradesh and Himachal Pradesh. Commercial citrus cultivars grown in different regions require different soil and climate conditions to thrive. It demonstrates wide adaptability of Citrus to wide range of soil and climatic conditions. The problems of various citrus cultivation belts differ according to region. The disease has a global distribution, affecting all citrus varieties in tropical and subtropical Citrus production regions, especially in warm, humid climates. Root rot and gummosis caused by *Phytopthora parasitica* var. *nicotianae* have been reported as a major constraint in maintaining optimum production in citrus, resulting in a 46% reduction in annual plant yield). It is responsible for 10-30% yield loss in citrus cultivation around the world (Timmer et al 2000). Naqvi (2003) recorded 20% yield losses in the citrus industry in Central India. Potassium salt of phosphonic acid, also known as potassium phosphonate (H_3PO_3), have been promoted and used as resistance inducers as well as direct inhibitors of oomycetes growth and sporulation with lower risk to human health and the environment when compared to conventional fungicides (Kromann et al 2012). Potassium phosphonate has a high level of symplastic ambimobility or movement in both xylem and phloem. Translocation in phloem allows the chemical to move from leaf tissues to the crowns and roots . Histological and biochemical studies confirmed that potassium phosphonate application increased host resistance to pathogen invasion (Jackson et al 2000, Daniel and Guest 2006). Previously, the efficacy of potassium phosphonate against black pepper foot rot (*Phytophthora capsici*) and Arecanut nut rot (*Phytophthora arecaea*) was determined in India (Lokesh et al 2012 and Hegde 2015). However, information on the use of potassium phosphonate for the management of *Phytophthora* root rot and gummosis diseases in mandarin is needed. In this study, the effects of potassium phosphonate and conventional fungicides on the development of root rot and gummosis were investigated in four years of full-scale field trials in three Indian states (Maharashtra, Punjab and Assam).

MATERIAL AND METHODS

The field trial was conducted at multi-locations, at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Maharashtra) on variety Nagpur mandarin; the Fruit Research Farm, Punjab Agricultural University, Ludhiana

(Punjab) on variety Kinnow mandarin and the Citrus Research Station, Tinsukia (Assam), on variety Khassi mandarin. The research trial was in Randomized Completely Block Design with eight treatments and three replicates (Table 1). The fungicides, bio-agents, and potassium phosphonate were applied twice at 45-day intervals during July to September 2018-19 to 2020-21. Fosetyl-Al @0.2% was used as a standard check, and an untreated control (absolute check) was also used. Bordeaux paste is commonly used on tree trunks up to 45-60cm above the soil level in all treatments except control as pre and post monsoon. Respective doses of fungicides and bio-agents were applied to the plants' foliar and soil regions in the appropriate amounts. The observations on trunk lesion size recovery, canopy volume, feeder root rot rating, soil disease potential and number of fruits per tree were recorded. The residue analysis was also conducted.

The trunk lesion size was noticed on the experimental plants (main stem/side branches) before and after the second the application of treatments.

Trunk lesion size recovery = (Initial trunk lesion size - Final trunk lesion size)/Initial trunk lesion size x 100

The feeder root rot rating was determined by using Grimm and Hutchinson's scale (1973). The root rot rating scale (1-5) was: 1= No visible symptoms, 2= A few roots with symptoms (1-25%), 3= Majority of roots with symptoms (26-50%), 4= All roots infected, cortex sloughed from major roots (51-75% rotted), and 5= Majority roots dead or missing (>76% rotted). The canopy volume was estimated using the formula proposed by Westwood et al (1993), and the increase canopy volume was calculated.

Percent increase in canopy volume= (Final canopy volume -Initial canopy volume)/ Initial canopy volume X 100.

The soil disease potential of *Phytophthora* soil population was estimated by serial dilution of soil and using specific bait to trap *Phytophthora* according to procedure described by Grimm and Alexander (1973). Fruit samples for residue analysis were collected from 03 months after the second application and sent to the Pesticides Residue Analysis Laboratory at the National Horticultural Research and Development Foundation, Nasik (Maharashtra). Fungicides residues in fruits samples were quantified using the Liquid Chromatography with tandem Mass Spectrometry (LCMS/MS) technique. The experimental data were subjected to analysis for variance for various treatments by using WASP 2.0 software.

RESULTS AND DISCUSSION

The trunk lesion size was measured before the first application of treatments, and the final trunk lesion size was measured 45 days later. There were significant differences in trunk lesion size recovery compared to control observed with different concentration of fungicides and bio-agent's application (Table 2). Among the different treatments, foliar application of potassium phosphonate @ 3g/liter at 45 days' interval significantly exhibited the maximum percent trunk lesion size recovery (51.54%) over the three locations and was at par with treatment T5 (dimethomorph (50WP) + mancozeb (75WP) {1g + 2g/ l water tank mix} + *T. harzianum* + *P. fluorescens* (each 100g/plant with FYM 1kg 25 days of interval) and T7: (Fosetyl -Al @0.2%) where it was 44.17 and 42.47%, respectively. In the absolute control, there was no tree trunk recovery of oozing lesions recorded. It is clearly demonstrated that the application of fungicides and bio-agents through foliar and soil combinations aids in the reduction of lesions oozing from tree trunks.

Subsequent to final application of treatments, feeder root rot index, which included both infected and healthy roots, was observed from the basin of treated plants and was found in the range of 1.07 to 3.12 (Table 2). The untreated control had the highest feeder root rot index (3.12). The lowest feeder root rot index (1.07) was in treatment T6 (Foliar spray of potassium phosphonate @ 3 g/l water). The results showed that the respective fungicides and bio-agents were effective in reducing pathogen infections in the roots. The soil disease potential index is expressed as the highest dilution at which

Table 1. List of fungicides and bio-agents used in study

Treatment	Trade name	Formulation
Cymoxanil 8 % + Mancozeb 64 % @2.5g/l	Curzate M8	Combi product in WP formulation
Dimethomorph 50 WP @ 1g/l + Mancozeb 75 WP 2g/l	Lurit and Dithane M45	Both in WP formulation (Tank mix application)
Trichoderma harzianum(10^7CFU/g) 100g/tree	Respective University formulations	Talc powder base formulation 100 g each mix with 1 kg FYM
Pseudomonas fluorescence(10^8CFU/g) 100g/tree		
Potassium phosphonate (Potassium salt of phosphonic acid 98% Inert compounds 02%)	Chemical grade powder	Water soluble powder base formulation
Fosetyl –Al 80%	Aliette	WP formulation

sporangia are observed. Treatment T6, had the lowest soil disease potential (12.83). This was followed by treatment T5 and T2 and T3 soil application of *Trichoderma harzianum* + *Pseudomonas fluorescens* (100g/plant each) with carrier material of FYM 1kg and T4 with carrier material of FYM 1kg where soil disease potential was recorded 15.89, and 17.11, respectively (Table 3). Maximum soil disease potential was recorded in untreated control (63.17).

It is evident from the pooled data of all the locations that among all the treatments, the maximum canopy volume increase (7.93%) was in T6, where foliar spray of potassium phosphonate @ 3g/l water was undertaken (Table 3). Treatment T7 was the next best treatment, with increase in canopy volumes of 6.79. The maximum fruit yield of 17.84 t/ha was in the plots sprayed with foliar spray of potassium phosphonate @ 3g/l water (T6), The minimum yield of 9.43 t/ha was recorded in the untreated plots (T8) i.e. absolute control (Table 4).

Economics of the treatments was computed by considering the additional cost of applying treatments only (Table 4). The highest incremental cost benefit ratio (ICBR) 4.01 was in treatment T6 (Twice application of potassium phosphonate @ 3g/l water). Residue analysis was performed, and in treatment T1, T2, T4, T5, and T7, residues

Table 2. Effect of various treatments on recovery from trunk lesion and feeder root rot index of citrus (Pooled data for 4 years)

Treatments	Trunk lesion size recovery (%)			Pooled mean	Feeder root rot index			Pooled mean
	Akola	Ludhiana	Tinsukia		Akola	Ludhiana	Tinsukia	
T1: Cymoxanil + mancozeb (8 + 64 WP) 2.5g/l water (combi product)	26.62	45.92	35.90	36.15	1.67	2.00	1.59	1.75
T2: Dimethomorph (50WP) + mancozeb (75WP) (1g + 2g/ l water tank mix)	28.92	40.73	40.42	36.69	1.50	3.00	1.28	1.93
T3:*T. harzianum* + *P. fluorescens* (100g/plant) with FYM 1kg	22.20	29.28	27.10	26.19	1.70	3.00	1.57	2.09
T4: T1 followed by T3 after 25 days interval	32.86	52.57	33.17	39.53	1.20	2.00	1.32	1.51
T5: T2 followed by T3 after 25 days interval	33.77	48.65	50.10	44.17	1.13	2.00	1.15	1.43
T6: Potassium phosphonate @ 0.3% foliar spray (two spray pre and post monsoon)	38.26	65.55	50.80	51.54	0.93	1.00	1.27	1.07
T7:Fosetyl -Al 0.2% (two spray pre and post monsoon)	34.16	62.36	30.89	42.47	1.00	1.00	1.44	1.15
T8: Control	0.00	0.00	0.00	0.00	2.50	4.00	2.86	3.12
CD (p= 0.05)	7.45	12.46	7.92	11.95	0.24	1.04	0.32	0.72

Table 3. Effect of various treatments on soil disease potential and canopy volume increase (Pooled data for 4 years)

Treatments	Soil disease potential			Pooled mean	Canopy volume increase %			Pooled mean
	Akola	Ludhiana	Tinsukia		Akola	Ludhiana	Tinsukia	
T1	25.50	22.33	32.0	26.61	3.56	7.68	3.90	5.05
T2	24.63	23.00	32.0	26.54	3.68	5.63	3.81	4.37
T3	26.25	27.67	32.0	28.64	2.69	3.66	1.78	2.71
T4	19.00	16.33	16.0	17.11	5.60	9.68	3.26	6.18
T5	20.00	17.67	10.0	15.89	6.43	8.23	4.64	6.43
T6	18.50	10.00	10.0	12.83	7.81	11.23	4.75	7.93
T7	21.50	10.33	32.0	21.28	7.07	10.61	2.70	6.79
T8	63.50	62.00	64.0	63.17	0.93	-	0.53	0.49
CD (p= 0.05)	9.38	16.66	6.42	8.83	0.31	1.03	0.51	2.68

See Table 1 for treatment details

were below the quantification level. In treatment T6, where a chemical spray of potassium phosphonate @ 3 g/l water foliar spray was applied with peel 0.547mg/kg and without peel 0.275 mg/kg residue was observed. However, that was below the MRL (90 mg/kg). When compared to the control and different fungicides, the application of potassium phosphonate significantly increased the tree trunk lesion size recovery from oozing lesions. After Bordeaux paint and treatments applications, trees with gummosis symptoms initiated signs of recovery. The recovery was manifested as new flushes that turned dark green and grew normally. The use of Bordeaux paste painted trees and potassium phosphonate foliar application resulted in faster and more recovery from trunk lesions. It appears that gummosis affected trees recovered from infections and were able to absorb proper nourishment for their development after treatment application. The tree in the control plots had small chlorotic flushes, twig dieback, gummosis and bear few number of fruits. This could attribute to poor growth and disruption of normal transport in gummosis-infected plants (Gade and Koche, 2012).

Foliar applications of potassium phosphonate induced a systemic defense response in plants, including an increase in phytoalexins, lignin and chitinase contents as well as enhanced peroxidase and polyphenol-oxidase activities as part of the phosphonate induced defense mechanism (Smith *et al.,* 1997). Treatment T6(Foliar spray of potassium phosphonate @ 3g/l water) had the highest increase in canopy volume (7.93%) and the highest yield (17.84 t/ha). Canopy volume represents plant growth in response to continuous nutrient uptake without affecting any deviation in plants. *Phytophthora* infections in mandarin plants cause a gradually decline in foliage due to decay of feeder roots and oozing of gums from the tree trunk. The highest tree trunk lesion recovery, the least feeder root rot, and the least soil disease potential of *Phytopthora* sporangia were recorded in treatment T6, which resulted in an increase in canopy volume and provided the highest fruit yield.

The observations were in conformity with those of Hegde and Mesta (2014), who reported that spraying with potassium phosphonate @ 6 ml/l and soil drench @ 4 ml/l water reduced the incidence of pod rot caused by *Phytophthora theobromae* in cocoa. Similarly, Ingle et al (2020) found that treating infected mandarin plants with foliar spray + soil drenching of potassium phosphonate @ 3 ml/l water resulted in a lower average number of lesion with oozing (28.39%), a minimum feeder root rot index (2.17), an increase in canopy volume (11.15%), and a higher fruit yield (65.89 kg/ per tree). They also reported that the potassium phosphonate at three different concentrations against *P. nicotianae in vitro* and found that the potassium phosphonate was effective in arresting *P. nicotianae* growth, with complete (100%) inhibition observed in tested doses. The results were also in accordance with those described by Lokesh et al (2012) in respect of potassium phosphonate @ 0.3 % as spraying and drenching with soil application of *Trichoderma harzianum*, @ 50 g/vine along with neem cake (1 kg/vine) to the black pepper vines against *Phytophthora* foot rot being highly effective in reducing the population of *Phytophthora* and increasing yield when compared to the farmers practice with use of 1 % Bordeaux mixture as spray. Furthermore, the current findings are in agreement with the report of Hegde (2015), where potassium phosphonate effectively protected areca nut plants from *Phytophthora arecae* -induced nut rot disease. In comparison to fosetyl-Al, potassium phosphonate applied as a foliar spray or soil drench resulted in less *Phytophthora citricola* stem infection of *Persea indica* seedlings (Fenn and Coffey 1987).

Table 4. Effect of various treatments on fruit yield and economics of treatments (pooled data for 4 years)

Treatments	Fruit yield (t/ha)			Pooled mean	ICBR			Pooled mean
	Akola	Ludhiana	Tinsukia		Akola	Ludhiana	Tinsukia	
T1	15.66	15.22	16.07	15.65	2.32	2.53	1.73	2.19
T2	15.97	14.84	18.09	16.30	2.20	2.35	1.86	2.14
T3	14.46	11.83	15.23	13.84	0.46	1.88	1.56	1.30
T4	16.46	16.16	17.75	16.79	1.96	3.74	1.83	2.51
T5	17.14	15.56	18.19	16.96	2.60	3.07	2.13	2.60
T6	18.16	16.94	18.42	17.84	5.78	4.12	2.14	4.01
T7	17.30	16.36	14.61	16.09	4.30	3.80	1.64	3.25
T8	13.31	7.68	7.30	9.43				
CD (p= 0.05)	1.08	3.10	2.53	2.52				

See Table 1 for treatment details

The potassium phosphonate quickly absorbed and translocated in the xylem then moves into and translocated in the phloem, where its distribution is subjected to normal source sink relationship in the plants. The radioactive ^{32}P was used to demonstrate the translocation of phosphonate to different parts of the black pepper plant (Anil Kumar et al 2009). Graham (2011) also demonstrated that potassium phosphonate is highly systemic, rapidly absorbed by leaves and transported to fruits, where it protects against citrus brown rot *caused* by *Phytophthora palmivora*. By disrupting pathogen metabolism and activating their own defense mechanisms, potassium phosphonate-treated plants appeared to be capable of creating an antimicrobial environment more effectively than non-treated chemical fungicide plants (Daniel and Guest 2006). The active constituent working against the pathogen is phosphonate (phosphate) or phosphonic acid, which is a dynamic component of this chemical within plants. According to Lovatt (1998), a single foliar application of phosphonate promoted agronomically important traits in citrus and avocado, such as fruit size, fruit yield, anthocyanin content, floral intensity, and total soluble solids. The effects of phosphonate on fruit production and quality have been documented (Lovatt, 1999 and Rickard 2000) for example, foliar sprays improved orange tree yield.

Potassium phosphonate residue was determined using LCMS/MS on fruit samples with peel, and the results confirmed that phosphonic acid was present in treated plants. Potassium phosphonate has been found to be remarkably persistent in plants. Using high performance liquid chromatography (HPLC) and ion chromatography, Saindrenan et al (1985) and Fenn and Coffey (1989) determined the phosphate and phosphonate concentrations in higher plants. Potassium phosphonate has high phloem mobility, and is translocated to various parts of the plant including the tubers (Cohen and Coffey 1986). Even after three months, potassium phosphonate residues were found in fruits from plants treated during the fruit bearing season. Concentrations of potassium phosphonate in fruits can be used safely at levels lower than the MRL (the MRL for phosphonic acid in the EU is 90mg/kg for citrus fruits; EFSA 2021).

CONCLUSIONS

The disease has significantly reduced with increase in trunk lesion recovery, maximum per cent canopy volume, highest fruit yield, and lowest soil disease potential in potassium phosphonate (0.3%). This potassium phosphonate based management technology is more ecologically friendly than conventional management practices, resulting in economic benefits for farmers.

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