J. Hortl. Sci.
Vol. 15(2) 153-160 : 2020

Original Research Paper

Evaluation of potassium salt of phosphonic acid in Nagpur mandarin with special reference to *Phytophthora* management

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ABSTRACT

***Phytopthora parasitica* var. *nicotianae* is a major fungal pathogen that causes foot rot, root rot, crown rot, gummosis, leaf fall and brown rot diseases in Nagpur mandarin in the entire Vidarbha region of Maharashtra. For the efficient management of root rot and gummosis due to *Phytophthora*, potassium salt of phosphonic acid (PSPA) was evaluated under field and laboratory conditions. In field trials, infected plants were treated with different concentration of PSPA by foliar spray and soil drenching. The results revealed that foliar spray + soil drenching of PSPA at 3 ml/liter water was better with respect to the average reduction in no. of lesion with oozing (28.39%), minimum in feeder root index (2.17), increase in canopy volume (11.15%) and higher fruit yield (65.89 kg/ per tree). Effect of PSPA was assayed at three difefernt concentarions against *P. nicotianae* under *in vitro*. PSPA was found most effective in arresting growth of *P. nicotianae* as complete (100%) inhibition obsereved in tested doses.**

Keywords: Foot rot, Gummosis, Nagpur mandarin, *Phytophthora* and Potassium salt of phosphonic acid (PSPA)

INTRODUCTION

Mandarins (*Citrus reticulata* Blanco) occupy a place of prime significance among the major fruit crops of India positioning third after mango and banana. They are a good source of vitamin C, as well as several other vitamins, minerals, and antioxidants. As per third advance estimates 2019-20, total land under orange (Mandarin orange/kinnow) cultivation in is 4.79 lakh hectare with production of 63.97 lakh tonnes (Anon., 2020). Major states engaged in orange cultivation are Madhya Pradesh, Punjab, Maharashtra, Rajasthan and Haryana.

Phytophthora spp. infect citrus plants at all stages and may infect most parts of the plant, including roots, stem, branches, twigs, leaves and fruits. Root rot, foot rot (also known as "gummosis", "trunk gummosis" or "collar rot"), fruit brown rot, twig and leaf dieback (often indicated collectively as "canopy blight") and rot (better known as "damping off") of seedlings, all incited by *Phytophthora* spp., may be considered diverse faces of the alike disease (Naqvi, 2000). In citrus, gummosis and foot rot (*Phytopthora parasitica* var. *nicotianae*) is reported as major constraint to sustain optimum production and it reduces yield by 46 per cent annually (Menge, 1993). It is responsible for 10-30 per cent yield loss in citrus cultivation around the world (Timmer *et al.,* 2000). The disease is also reported to pose a serious problem in mandarin grown in on large scale in Vidarbha region of Maharashtra (Naqvi, 2003). The severity of the disease is higher during monsoon season. Integrated disease management package that incorporates fungicides, biocontrol agents and organic amendments is required. Number of workers (Thind *et al*., 2004; Gade *et al.,* 2005; Kaur *et al.,* 2009; Jagtap *et al.,* 2012; and Singh *et al.,* 2015) used contact and systemic fungicides and bioagents for management of root rot/gummosis disease due to *Phytophthora* in citrus crops. Use of conventional fungicides can moderate the problem up to some level but cannot eliminate it; moreover, there are chances of resistance risk in the pathogen due to the use of systemic chemicals.

Potassium salt of phosphonic acid is chemically known as potassium phosphonate (H_3PO_3). The dynamic component of this chemical within plants is phosphonate (phosphate) or phosphonic acid which is the active constituent working against the plant pathogen (Fenn and Coffey, 1987; Dunhill, 1990 and Guest and Grant, 1991). PSPA possess significant symplastic ambimobility or movement in both xylem and phloem (acropetally and basipetally). Translocation in phloem allows the chemical to move from leaf tissues to the crowns and roots (Ouimette and Coffey, 1990). Confirmation from histological and biochemical studies prove that PSPA application increases level of host resistance to pathogen invasion (Jackson *et al.,* 2000 and Daniel and Guest, 2006). Previously in India, effectiveness of PSPA against foot rot of black pepper incited by *Phytophthora capsici* (Lokesh *et al*., 2012) and nut rot disease in areca nut (*Phytophthora arecaea*) have been evaluated (Hegde, 2015) and was found effective.

Table 1. Treatment details

Sl. No.	Treatments	Dose (ml/ L of water)
1	Foliar spray of PSPA	02
2	Foliar spray of PSPA	03
3	Foliar spray of PSPA	04
4	Soil drenching of PSPA	02
5	Soil drenching of PSPA	03
6	Soil drenching of PSPA	04
7	Foliar spray+Soil drenching of PSPA	03 + 03
8	Foliar spray of Fosetyl -Al	02 g
9	Absolute control	-

First foliar spray and soil drenching was given in September and succeeding second application at one-month interval (October)

However, there is lack of information on use of PSPA for management of the Phytophthora root rot and gummosis disease in Nagpur mandarin. Therefore, an effort was made to explore the efficacy of PSPA in managing Phytophthora root rot and gummosis disease of Nagpur mandarin in the endemic region of Vidarbha in Maharashtra.

MATERIALS AND METHODS

The research trial was conducted on Nagpur mandarin at Dr. PDKV, Akola in randomized block design with nine treatments and three replicates. Application of respective potassium salt of phosphonic acid (PSPA) (trade name Sanchar 40) was given in one-month interval during September and October 2018. Fosetyl - Al @ 0.2% was used as standard check and (Table 1). Individual dosages were applied at foliar and basin region of plants. The observations on number were lesions with oozing, canopy volume, feeder root rot rating, number of fruits per tree and phytotoxicity were recorded.

Number of oozing lesions was recorded on experimental plants (main stem/side branches) before the application of PSPA and after second application of PSPA. Reduction in oozing lesion measured by using following formula-

= (Initial no. of oozing lesion - Final no. oozing lesions / Initial no. of oozing lesion x 100

The feeder root rating was recorded before the application and after second application of PSPA. The feeder root rotting using scale (1-5) given by Grimm and Hutchinson (1973) and Gade *et al.* (2005) was followed Root scale (1-5): 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), 5= Majority roots dead or missing (>76% rotted). The canopy volume was as per the formula suggested by Westwood (1993) and increase in plant volume calculated as per the formula given below-

Increase in canopy volume (%)= (Final canopy volume-Initial canopy volume)/ Initial canopy volume X 100.

Observations on phytotoxicity symptoms were recorded for all treatments visually as per the guidelines of Central Insecticide Board, Govt. of India on 0–10 scale, 1-1 to 10%, 2-11 to 20%, 3-21-30%, 4-31-40% 5- 41-50% 6-51 to 60%, 7-61 to 70%, 8-71-80%, 9-81-90% and 10-91-100%. Effect on crop health *viz.*, leaf yellowing, tip necrosis, scorching, epinasty and hyponasty etc., were recorded on 0, 1, 3, 5, 7 and 10 days after application of each spray using the following score and per cent effect was worked out as per the method proposed by Nishantha *et al.* (2009).

In vitro study was also conducted to know the inhibition of pathogen by poison food technique (Nene and Thapliyal, 1993). PSPA was added to cornmeal agar at various concentrations A 6 mm diameter agar disk, taken from an actively growing fungal colony on agar without PSPA, was placed with pathogen side downward at the center of the plate (9 cm diameter). Plates were incubated in the incubator at 25 ±1 ^{0}C for ten days. Radial growth of pathogen recorded and per cent inhibition in each treatment was calculated by using formula of Vincent (1927).

Sample of fruits for residue analysis (harvested 3 months after second application) were sent to Pesticides Residue Analysis Laboratory, National Horticultural Research and Development Foundation, Nasik. Residue of PSPA in fruits samples were quantified by utilizing LCMS technique (Saindrenan *et al*., 1985).

The data collected from the experiments were subjected to analysis of variance for different treatments. Fisher's protected critical difference (CD) test was used to indicate the difference between the treatments at the probability level of $p < 0.01$ following the procedure described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The number of oozing lesions before the first application of PSPA and after second application of one-month interval and final oozing lesions were counted. There were significant differences in the in number of oozing lesions in plates treated with PSPA at different concentration of (Taldiz) and Fosetyl- Al. The results revealed that amongst the different treatments, foliar and soil drenching of PSPA @ 3ml/ liter at one-month interval significantly reduced the number of oozing lesions (8.75) on tree trunk with enhanced reduction in number of oozing lesions (28.39%). It was on par treatments (T3, T5, T6, T7). Maximum number of oozing lesions was recorded in absolute control (14.04). This clearly indicates that by the application of PSPA through soil or foliar and combinations will reduce no. with oozing lesions from tree trunk. There was high reduction with T7 compared to other treatments (Table 2).

Subsequent to final application (2nd foliar and drenching) feeder root rot index (infected and healthy effective roots) observed from the basin of treated plants and was found in the range of 2.17 to 2.97 (Table 2).

Table 2. Efficacy of potassium salt of phosphonic acid on oozing lesion and feeder root index

Sl. No.	Treatments	Dose Product (ml/L of water)	Number of Oozing lesion		Reduction in oozing lesion (%)	Feeder root Index	Feeder root index reduction over control (%)
			Initial	Final			
1	Foliar spray of PSPA	02 ml/L	12.18	9.78	19.68	2.33	21.55
2	Foliar spray of PSPA	03 ml/L	11.73	8.98	21.60	2.30	22.56
3	Foliar spray of PSPA	04 ml/L	11.69	8.87	23.27	2.27	23.57
4	Soil drenching of PSPA	02 m/L	12.25	9.90	19.86	2.23	24.91
5	Soil drenching of PSPA	03 ml/L	11.70	8.87	22.64	2.20	25.92
6	Soil drenching of PSPA	04 ml/L	11.67	8.79	23.93	2.20	25.92
7	Foliar spray + Soil drenching of PSPA	03 ml/L + 03 ml/L	12.25	8.75	28.39	2.17	26.93
8	Foliar spray of Fosetyl -Al @ 02 g/L	02 g/L	12.75	10.37	19.30	2.30	22.56
9	Absolute control	—	11.93	14.04	-	2.97	
	SE (m)±		-	0.43		0.12	
	(CD at 5%)		NS	1.29		0.36	

Maximum extreme feeder root rot (2.97) index was recorded in control treatment. Highest per cent reduction (26.93%) in feeder root rot index over control was observed in treatment T7 (Foliar spray + Soil drenching of PSPA @ 3ml/L each) followed by T6 and T5 (25.92%) and T4 (24.91%). The results indicated that concentrations and application methods were able to reduce roots infections of pathogen.

Maximum canopy volume (10.33 m^3) with an increase by 11.15% was recorded in treatment T7 (Foliar spray + Soil drenching of PSPA @ 3ml/L). Next best array of treatments was T6, T3, T5, and T2 that recorded higher canopy volume 10.17,9.83, 9.76 and 8.99 m^3 with increase in canopy volume 7.96, 6.46, 5.63 and 5.19 respectively (Table 3).

The maximum yield of (65.89 kg/tree) was registered in the plots sprayed with Foliar spray + Soil drenching of PSPA @ 3ml/L (T7), which was par with 62.11 kg/tree in the plots soil drenched with PSPA @ 4ml/L (T6), foliar spray of PSPA @ 4ml/L (T3) 60.78 kg/tree and plots drenched with PSPA @ 3ml/L (T5) 59.78 kg/tree (Table 3). Commenly used fungicides and standard check Fosetyl-Al recorded yield 59.33 kg/tree (T8) which was notably lower in compared to PSPA application. The lower yield of 51.11 kg/tree was recorded in the untreated plots (T9) i.e., absolute control.

Table 3. Efficacy of potassium salt of phosphonic acid on canopy volume and fruit yield

Sl. No.	Treatments	Dose Product (ml/L of water)	Canopy volume (m^3) Initial	Canopy volume (m^3) Final	Increase in canopy volume (%)	Fruit yield (kg/tree)
1	Foliar spray of PSPA	02 ml/L	8.50	8.91	4.82	57.78
2	Foliar spray of PSPA	03 ml/L	8.55	8.99	5.19	59.56
3	Foliar spray of PSPA	04 ml/L	9.23	9.83	6.46	60.78
4	Soil drenching of PSPA	02 m/L	8.68	9.12	5.03	58.22
5	Soil drenching of PSPA	03 ml/L	9.24	9.76	5.63	59.78
6	Soil drenching of PSPA	04 ml/L	9.42	10.17	7.96	62.11
7	Foliar spray + Soil drenching of PSPA	03 ml/L+ 03 ml/L	9.29	10.33	11.15	65.89
8	Foliar spray of Fosetyl -Al @ 02 g/L	02 g/L	9.35	9.90	5.92	59.33
9	Absolute control	-	8.51	8.56	0.59	51.11
	SE (m)±		-	0.37		2.25
	(CD at 5%)		NS	1.11		6.38

During the course of investigation phytotoxicity symptoms not observed in any of the treatments at respective days of observation.

Efficacy of PSPA at respective concentration was tested *in-vitro* by following poison food technique for mycelial growth of *Phytophthora nicotianae.* After 10 days of inoculation, PSPA was found most effective in arresting complete growth of *P. nicotianae* as complete (100%) inhibition obsereved in tested doses i.e., 2, 3, and 4 ml/L. Radial growth of 20.32 mm was recorded in control plate on 10th day (Table 4).

Residue examination of treatment T3 (PSPA foliar shower @ 4ml/L) and T7 (Foliar spray + Soil drenching of PSPA @ 3ml/L) was done (Table 5). Treatment T3 recorded 0.329mg/kg residue content in harvested fruits however in treatment T7 recorded 0.666 mg/kg residue PSPA was observed that were below the recomond of 70g/kg level of MRL (according to EU).

Table 5. Residue analysis of PSPA

Sl. No.	Treatments doses	Equipment used	LOQ (mg/kg)	Residue content (mg/kg)	Fruits parts used
1	PSPA foliar spray @ 4ml/L	LCMS	0.010	0.329	Fruits with Peel
2	PSPA foliar spray @ 3 ml/L + Soil drenching @ 3 ml/L	LCMS	0.010	0.666	Fruits with Peel

Results demonstrated that application of PSPA significantly reduced the number of oozing lesions compared to chemical fungicide (Fosetyl -Al) at different concentrations. The results were also consistent with feeder root index, increase in canopy volume and fruit yield. Among the different concentrations and application methods of PSPA, two applications of PSPA @ 3ml/L foliar + soil drenching (one moths interval) significantly reduced the gummosis symptoms (28.39%) i.e., reductions in number of oozing lesions, reduced feeder root not index (Phytophthora root rot symptoms) (26.93%), increase in canopy volume (11.15%), and higher yield (65.89 kg/tree).

zone Use of PSPA at foliar plant and root challenge the infection adequately and reduce the gummosis and root rot. This had been observed by Hegde and Mesta (2014) who reported that in cocoa, spraying with PSAP @ 6 ml/L and soil drench @ 4 ml/L had reduced the incidence of pod rot caused by (*Phytophthora theobromae)*. Lokesh *et al*. (2012) also reported that application of potassium phosphonate @ 0.3 % as spraying and drenching with soil application of *T. harzianum*, @ 50 g/vine along with neem cake (1 kg/vine) to the black pepper vines against Phytophthora foot rot served as best treatment when compared to the farmers practice with use of 1 % Bordeaux mixture as spray. Moreover, current results are in agreement with the report of Hegde (2015) where potassium phosphonate effectively protected areca nut plants against nut rot disease incited by (*P. arecae)*. Compared to Fosetyl -Al, potassium phosphonate applied as a foliar spray or soil drench reduced stem infection of *Persea indica* seedlings by *Phytophthora citricola* (Fenn and Coffey, 1987). Numerous reports confirm that PSPA is readily absorbed by leaves and roots (Groussol *et al*., 1986; Schroetter *et al*., 2006 and Graham, 2011). After application of PSPA on the plant, the chemical gets translocated upwards in the xylem and downwards in the phloem (Guest and Grant, 1991). Its translocated in the phloem and its distribution is then subjected to normal source sink relationship in the plants. The translocation of phosphonate to different parts of black pepper plant was demonstrated by using radioactive ^{32}P Kumar *et al*., 2009). Graham (2011) also experimentally proved that potassium phosphonate is highly systemic rapidly taken up by leaves and to move to fruit and provide protection against citrus brown rot of fruit caused by *Phytophthora palmivora*. In adding together, PSPA treated plants appear to be capable to create an anti-microbial environment more effectively by disrupting pathogen metabolism and triggering their own defense mechanisms (Daniel and Guest, 2006). Niere *et al.* (1994) proposed that the toxicity of PSPA on oomycetes was due to an increased level of inorganic poly-phosphonate, which is known to inhibit key phosphorylation reactions in them. In addition, PSPA also found to alter the nucleotide pools and pentose phosphate metabolism in *Phytophthora citrophthora* (Barchietto *et al*., 1992).

In present experiment, phytotoxicity symptoms were not visually observed on treated plants in respective concentrations and on respective days of observations. These results are in line with Pilbeam *et al.* (2000) who did not observe any phytotoxicity symptoms in *Eucalyptus* spp. treated with different concentrations of phosphonate. Guest and Grant (1991) observed that PSPA caused minimal toxicity when used at acute concentrations. Foliar sprays of phosphonate can cause phytotoxicity to citrus leaves and rapidly growing fruit later in the season if applied at high rates, at high temperatures, or if the tree is under drought stress (Le Roux, 2000). During the experimentation, congenial conditions prevailed and no phytotoxicity symptoms was observed.

PSPA effectively inhibited P. *nicotianae.* Anti-fungal chemical effects of the PSPA against *Phytophthora* spp. were reported by Cohen and Coffey (1986); Fenn and Coffey (1984); Bompeix et *al*., 1989), Grant *et al.,* (1990) and Truong *et al.* (2012). Wong (2004) documented in his *in vitro* studies that phosphonate suppressed the growth rate of *Phytophthora capsici* by hyphal lysis.

Fruits samples with peel subjected for LCMS/MS for determination of PSPA residue and results confirmed that phosphonic acid present in treated plants. PSPA remarkably persistent in plants. Concentrations of PSPA in the fruits can be well below those MRL level (MRL level is 70g/kg of phosphonic acid as per EU).

Present results indicated that foliar sprays and soil drench of PSPA to Nagpur mandarin could be a practicable method of application for Phytophthora root rot and gummosis management.

ACKNOWLEDGEMENTS

Authors are grateful to AICRP on Fruits scheme for providing laboratory facility and resources for the experiment. All sort of assistance rendered by DOR, Dr. PDKV, Akola and Agri-Search (India) Pvt. Ltd., Nasik Maharashtra for the above study is gratefully acknowledged.

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(Received on 12.11.2020 and Accepted on 28.12.2020)