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Variability in the sensitivity of *Phytophthora capsici* isolates to potassium phosphonate

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ABSTRACT: Potassium phosphonate is recommended for the control of *Phytophthora* foot rot disease of black pepper (*Piper nigrum* L.). Sensitivity of 29 isolates of *Phytophthora capsici*, obtained from black pepper plants at various locations of Kerala and Karnataka, to potassium phosphonate was studied. There were significant variations EC_{50} and EC_{90} values for mycelial growth, sporulation, zoospore release and zoospore germination to different concentrations of potassium phosphonate. The EC_{50} values for mycelial growth, sporulation, zoospore release and zoospore germination ranged from 89.3µg/ml-603µg/ml, 0.25µg/ml- 36.3 µg/ml, 0.84 µg/ml to 29.9 µg/ml and 1.5 µg/ml-37.3 µg/ml, respectively. The EC_{90} values for mycelial growth were 573.5 µg/ml to 1635.20 µg/ml, for sporulation it was 2.1 µg/ml to 129.3 µg/ml, for zoospore release it was 14.2µg/ml to 72.7 µg/ml and for zoospore germination it was 5.6 µg/ml to 103.4 µg/ml. Among the four stages of life cycle of *P. capsici*, sporulation stage was the most sensitive to potassium phosphonate and mycelial growth was the least affected. Sporulation being an important event in the spread of epidemics, application of potassium phosphonate at critical time would be essential in preventing the spread of infection.

Key words: Black pepper, potassium phosphonate, *Phytophthora*, variability, sensitivity

The foot rot pathogen *Phytophthora capsici* is one of the major limiting factors in the production of black pepper (Anandaraj, 2000). The principal methods of controlling *P. capsici* include cultural practices and the use of fungicides. The current practices to combat foot rot pathogen include application of Bordeaux mixture, copper oxychloride and potassium phosphonate (Sarma and Anandaraj, 1997). Potassium phosphonate has shown to provide excellent control of a number of soil borne diseases caused by *Phytophthora* species (Cohen and Coffey, 1986; Guest and Grant, 1991; Ali *et al.,* 1996; Opoku *et al.,* 1998; Rajan, 2000 and Veena and Sarma, 2000). Potassium phosphonate is ambimobile and because of its exceptional mobility through phloem, it can be applied either as a foliar spray or as a soil application (Sarma *et al.,* 1988; NRCS, 1992). Potassium phosphonate is apparently benign and provide durable control. The effectiveness of potassium phosphonate against plant disease depends on the sensitivity of the pathogen to phosphonate (Guest *et al*., 1995). Even though much information on the sensitivity to potassium phosphonate of several *Phytophthora* species is available, very little is known for *P. capsici* infecting black pepper. *Phytophthora* species and isolates within a species are reported to vary in their sensitivity to fungicides (Coffey *et al.,* 1984; Ramachandran *et al.,* 1988). In the present investigation an attempt has been made to study the variation in sensitivity of various isolates of *P. capsici* to different concentration of potassium phosphonate.

MATERIALS AND METHODS

Twenty-nine isolates of *P. capsici* obtained from black pepper at different locations (14) of Kerala and (15) of Karnataka were used for the study. All the 29 isolates used in this study were from the collections maintained at National Repository of *Phytophthora*, Indian Institute of Spices Research (IISR), Calicut.

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Mycelial growth inhibition

The commercial formulation, Akomin-40 (Rallis India Ltd.) containing 40% phosphorous acid was used for the study. For the studies on growth of mycelium, poisoned food technique (Zentmyer, 1955) was used. Potassium phosphonate was sterilized by passing it through a 0.22 µm Millipore filter. Stock solution of the fungicide was prepared using sterile distilled water and appropriate quantities were incorporated to autoclaved carrot agar medium to form concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 µg/ml and dispensed into Petri plates. Three plates were maintained for each concentration. The test isolates were cultured on carrot agar for 48h and mycelial discs (1cm diameter) were cut from the growing edges and placed in the centre of plates containing carrot agar amended with various concentrations of the test chemical. The plates were incubated at 25±1°C, growth of colony was measured after 72 h. The radial growth of mycelium was measured at two points at right angle to each other from each of the three plates maintained for each concentration. The growth of the colony in control sets where no chemical was added was compared with that of various concentrations and the difference was converted into per cent inhibition.

Sporulation

The test isolates *P. capsici* were grown on carrot agar in the dark for 48 h at 25°C and 1cm diameter discs of mycelium

were cut and placed in Petri plates (3 plates/ concentration and 5 discs/plate). Potassium phosphonate at different concentrations (1, 5, 10, 20, 30 and 40 µg/ml) were placed on these discs so that each mycelial disc was partly submerged in fungicidal solution and incubated under continuous light for 48 h. In control, the discs were covered with sterile distilled water. The number of sporangia produced per microscopic field was counted. The average for six microscopic fields for each disc was counted and compared with that of control.

Zoospore release

The test isolates of *P. capsici* cultures grown on carrot agar medium for 48 h were taken and 1 cm diameter discs were cut and allowed to sporulate by pacing in water and incubating in continuous light for 48h. Such sporulating discs were taken in Petri plates, potassium phosphonate at different concentrations namely, 1, 5, 10, 20, 30 and 40 µg/ml placed over them and incubated at 4°C for 10 minutes. For each concentration three plates were kept with five discs in each plate. These plates were taken out and incubated at room temperature for 30 minutes. For control sets, sterile distilled water was used in place of potassium phosphonate. The number of sporangia germinated by releasing zoospores was counted. Six microscopic fields were observed for each disc (3 plates/concentration and 5 discs/plate) and per cent inhibition was calculated by comparing with control plates.

Zoospore germination

Sporulating discs of the test isolates of *P. capsici* were subjected to cold shock at 4°C for 10 minutes as described above and incubated at room temperature for 30 minutes for zoospore liberation. The zoospores present in 50 µl were mixed with 50µl fungicidal solutions so as to form final concentration of 1, 5, 10, 20, 30 and 40µg/ml and were placed on cavity slides. In the control, distilled water was used. All the slides were incubated at room temperature inside Petri plates lined with moist filter paper for 4 h. The numbers of zoospores present and number of them germinated were counted in 6 microscopic fields for each slide. Inhibition percentage was calculated by comparing with control. Linear regression analysis was performed to determine EC_{50} and EC_{90} values of different isolates. The EC_{50} and EC_{90} values were calculated from linear regression lines obtained by plotting the per cent inhibition of mycelial growth against the log concentration of potassium phosphonate.

RESULTS AND DISCUSSION

Considerable variability in the sensitivity of isolates to potassium phosphonate was observed for mycelial growth, sporangial production, zoospore release and germination.

Mycelial growth inhibition

All the 29 isolates showed differential response to potassium phosphonate. The EC_{50} and EC_{90} values for mycelial inhibition are given in Fig.1a. Of all the isolates tested, 97-53, an isolate obtained from infected leaf from Pulpally, Wynad showed the highest sensitivity (89.3 µg/ml) to potassium phosphonate and the isolate 96-5 obtained from the infected soil of Nettur, Idukki showed least sensitivity (603 µg/ml) to the chemical. Out of the 29 isolates, three isolates had EC_{50} values less than 100 µg/ml, three with value 100- 200 µg/ml, 15 isolates with 200-400 µg/ml and eight with value more than 400 µg/ml. All three sensitive

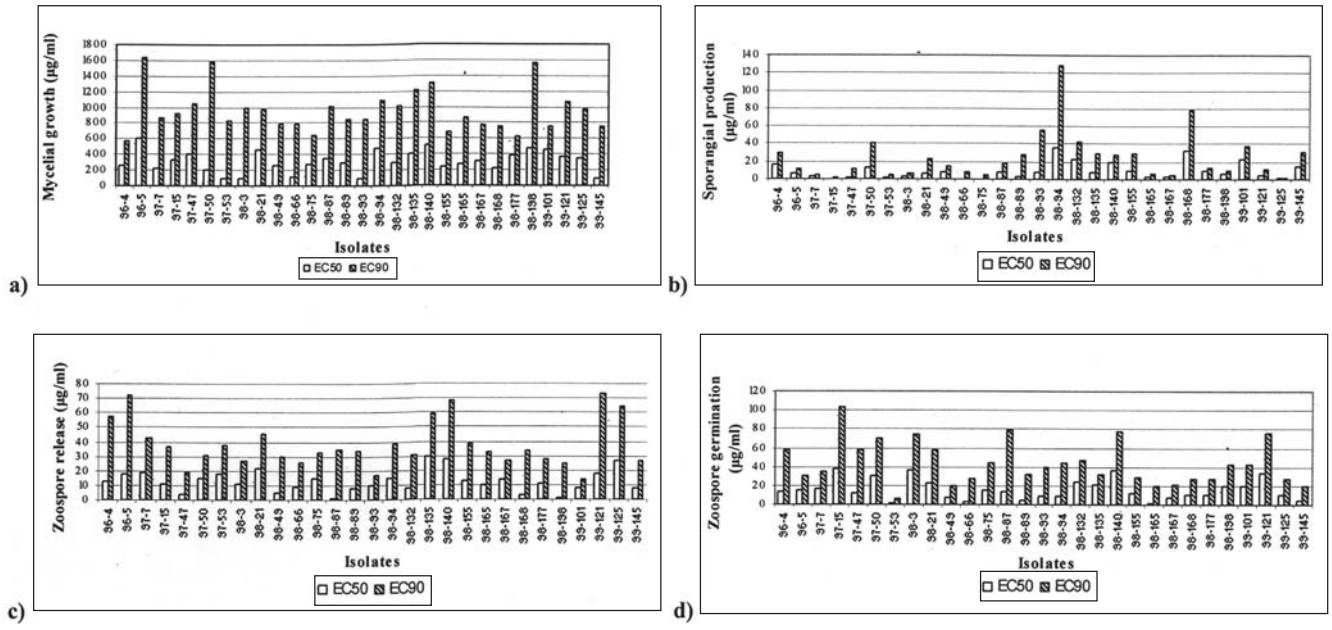

Fig. 1. Sensitivity of *Phytophthora* isolates to potassium phosphonate at various growth stages. (a) The EC_{50} for mycelial growth ranged from 89.3µg/ml to 603.0µg/ml and EC_{90} ranged from 573.5µg/ml to 1635.2µg/ml, (b) The EC_{50} for sporangial production ranged from 0.2µg/ml to 36.3µg/ml and EC_{90} ranged from 2.1µg/ml to 129.3µg/ml, (c)The EC_{50} for zoospore release ranged from 0.8µg/ml to 29.9µg/ml and EC_{90} ranged from 14.2µg/ml to 72.7µg/ml, (d) The EC_{50} for zoospore germination ranged from 1.5µg/ml to 37.3µg/ml and EC_{90} ranged from 5.6µg/ml to 103.4µg/ml

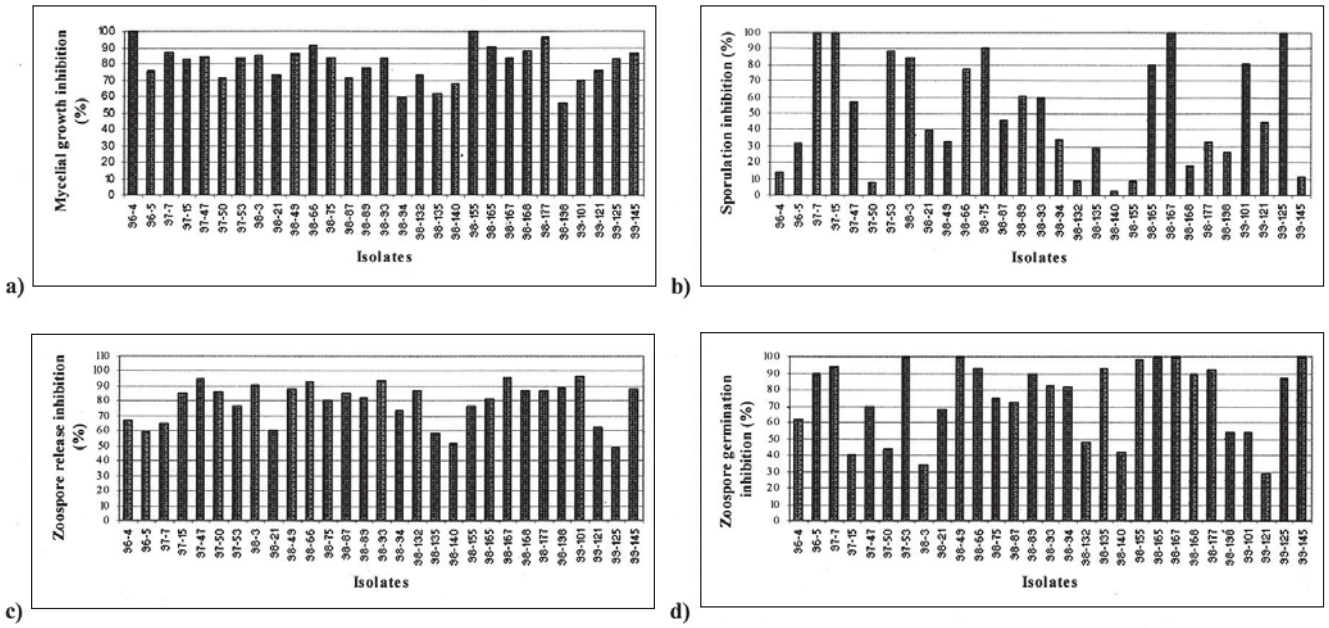

Fig. 2. Inhibition of growth, sporulation, zoospore release and zoospore germination at fixed concentrations of potassium phosphonate. (a) The inhibition of mycelial growth at 700 µg/ml, (b) The inhibition of sporulation at 5 µg/ml, (c) The inhibition of zoospore release at 30 µg/ml, (d) The inhibition of zoospore germination at 10 µg/ml

isolates were obtained from aerial portions, while the insensitive isolates from below ground, either soil or root. There was no correlation between geographical areas from which the isolates obtained and EC_{50} and EC_{90} values. Inhibition of sensitive isolates of *P. palmivora* has been directly related to the concentration of phosphate in its mycelium (Griffith *et al.,* 1993). In this study when all the isolates were grown at a fixed concentration of 700µg/ml mycelial growth inhibition varied among isolates. The inhibition ranged form 55.8% to 100% (Fig. 2a), which indicates that there can be a large variation in sensitivity among isolates to potassium phosphonate and their ability to accumulate phosphate in their mycelium.

Sporangial production

Among the four stages, the asexual reproductive phase involving sporangial production was most sensitive to potassium phosphonate. Coffey and Bower (1984) reported that the production of zoosporangia is especially sensitive to potassium phosphonate. The EC_{50} and EC_{90} values are shown in Fig. 1b. The most sensitive isolate 98-66, an isolate obtained from the infected leaves from Kodagu had EC_{50} value 0.2 µg/ml and EC_{90} value 8.72 µg/ml. Least sensitivity obtained with the isolate 98- 94 (EC_{90} value-129.3µg/ml). Out of the 29 isolates, 12 isolates (41.4%) had EC_{50} value less than 5 µg/ml, nine isolates (31.0%) with value 5-10 µg/ml and 4 (13.8%) each had with values between 10-20 µg/ml and more than 20 µg/ml (Fig. 1b). All the least sensitive isolates were obtained from Calicut district, Kerala. In general, soil and root isolates were less sensitive. The inhibition percentage calculated at 5µg/ml concentration showed differential response to potassium phosphonate and the inhibition ranged from 7.7% to 100% (Fig. 2b).

Zoospore release

The EC_{50} and EC_{90} values for inhibiting zoospores release are shown in Fig 1c. The most sensitive isolate was 98-198 (0.844 µg/ml) and the least sensitive was 98-135 (29.9 µg/ml). Four isolates (13.8%) had EC_{50} values less than 5µg/ml, seven (24.1%) between 5-10µg/ml, 14 isolates (48.2%) between 10µg/ml to 20µg/ml and four isolates (13.8%) had value more than 20µg/ml (Fig. 1c). The percentage inhibition of zoospore release at 30µg/ml ranged from 48.8% to 96.8% (Fig. 2c).

Zoospore germination

The EC_{50} and EC_{90} values are shown in the Fig. 1d. Five isolates (17.2%) had EC_{50} value less than 5 µg/ml and another five (17.2%) had value between 5-10 µg/ml and 11 (37.9%) had value between 10-20 µg/ml and eight (27.5%) required more than 20 µg/ml (Table 2). The percentage inhibition calculated at 10 µg/ml showed that inhibition varied from 28.7%-100% (Fig. 2d). Coffey and Bower (1984) opined that compared to spore production, germination of fungal propagules was less affected. The isolates were classified into sensitive and insensitive based on their EC_{50}

Table 1. Sensitivity of *Phytophthora* isolates to potassium phosphonate based on mycelial growth

Sensitive isolates EC_{50} < 100 (µg/ml)		In-sensitive isolates EC_{50} > 400 (µg/ml)	
Isolate No.	Source	Isolate No.	Source
97-53	Leaf	96-4	Soil
98-93	Leaf	97-47	Soil
99-145	Leaf	98-21	Soil
		98-94	Root
		98-135	Soil
		98-140	Soil
		98-198	Soil
		99-101	Root

Table 2. Sensitivity of *Phytophthora* isolates to potassium phosphonate on asexual reproductive characters

Stage of growth	Per cent distribution of isolates showing various sensitivity EC_{50} (µg/ml)			
	< 5.0	5.1-10.0	10.1-20.0	> 20.1
Sporangial production	41.4	31.0	13.8	13.8
Zoospore release	13.8	24.1	48.2	13.8
Zoospore germination	17.2	17.2	37.9	27.5

the four stages of the fungus, sporulation was the most sensitive. Potassium phosphonate has been reported to show low activity against mycelial growth of *Phytophthora* (Bompeix *et al.,* 1981). Coffey and Joseph (1985) and Dolan and Coffey (1988) reported that phosphonate inhibited sporulation at low concentrations without affecting mycelial growth. Production of zoosporangia is especially sensitive to potassium phosphonate (Coffey and Bower, 1984). Interference with the life cycle, by inhibition of sporulation or germination processes provides a window of opportunity

Table 3. In-sensitive isolates based on inhibition of mycelium and asexual characters

EC_{50} < 100 (µg/ml) Inhibition of mycelium		EC_{50} < 5 (µg/ml)					
		Sporangium production		Zoospore release		Zoospore germination	
Isolate no.	Source	Isolate no.	Source	Isolate no.	Source	Isolate no.	Source
97-53	Leaf	97-7	Leaf	97-47	Soil	97-53	Leaf
98-93	Leaf	97-15	Leaf	98-87	Leaf	98-66	Leaf
99-145	Spike	97-47	Soil	98-168	Stem	98-89	Leaf
		97-53	Leaf	98-198	Soil	98-165	Root
		98-3	Soil			99-145	Spike
		98-66	Leaf				
		98-75	Root				
		98-89	Leaf				
		98-165	Root				
		98-167	Root				
		99-125	Root				

values that were <100 µg/ml and 4003 µg/ml respectively. Among the sensitive isolates 97-53 (1.57 µg/ml), an isolate obtained from infected leaves from Pulpally and the least affected one was 98-140, a soil isolate from Sirsi (37.3 µg/ml). The sensitive isolates were from aerial parts and those obtained from roots and soil were insensitive to lower concentration of potassium phosphonate (Table 1). The isolates differed in their sensitivity to production of asexual reproductive structures. About 13.8% of the isolates had EC_{50} values >20 µg/ml for sporangial production and zoospore release and 27.5% for zoospore germination (Table 2). The present study indicates that high degree of variability exists among isolates of same species. The range of sensitivity to phosphonate found *in vitro* between different isolates is as great as the range observed between oomycetes and other fungi (Guest, 1984). Difference in variability of a sensitive isolate of *Phytophthora* was associated with the ability to take up the phosphonate anion (Dolan and Coffey, 1988). In a study involving sensitive and resistant isolates of *P. palmivora* Griffith *et al.* (1993) demonstrated that the resistant isolates were inhibited by phosphonate only when phosphate was limiting to growth and were able to exclude phosphonate effectively than sensitive isolates.

Present study indicated that there was no correlation between the sensitivity of the isolates and the geographical area of collection. The sensitivity for sporangial production, zoospore release and germination varied considerably. However, among the isolates studied one of the isolates 97-53 was most sensitive both for growth of mycelium, sporangial production and germination (Table 1, 3). Among for an antifungal compound. Fenn and Coffey (1984) has reported to have a close correlation between the *in vitro* and *in vivo* EC_{50} values for inhibition by phosphorus acid. *Phytophthora capsici* infections in black pepper occurs on all parts of the vine and the expression of symptoms depends up on cite of infection and extent of damage. The incidence of disease is severe during the wet monsoon period (Anandaraj, 2000). As the fungus is highly variable in its sensitivity to other fungicides such as metalaxyl (Ramachandran *et al.,* 1988) this variability in sensitivity has to be considered and the timing of application at the most appropriate period is crucial factor for formulation of management strategies.

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