Research Article

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Virulence of *Phytophthora* isolates from *Piper nigrum* L. and their sensitivity to metalaxyl-mancozeb

K.B. Vinitha, M. Anandaraj* and R. Suseela Bhai

Division of Crop Protection, ICAR-Indian Institute of Spices Research, Kozhikode-673 012, Kerala, India

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Abstract

Foot rot disease caused by *Phytophthora capsici* is the most destructive disease in black pepper. A total of 82 isolates from the National Repository of *Phytophthora* at ICAR-Indian Institute of Spices Research were evaluated for their sensitivity to metalaxyl-mancozeb and also tested for their virulence. Of the 82 isolates studied, 19 (23.2%) were highly sensitive to metalaxyl-mancozeb and 6 (7.3%) were comparatively insensitive (EC_{90} value >40 ppm; range 41.3 to 68.5 ppm). The isolates also varied in the degree of virulence, *viz.*, 34 (41.5%) were highly virulent, 39 (47.6%) moderately virulent, 4 (3.7%) mildly virulent, and 5 (6.1%) non-virulent. There was neither any significant correlation between sensitivity to metalaxyl-mancozeb and virulence nor with the geographical location.

Keywords: Fungicide, geographical location, pathogenicity, resistance, variability, virulence

Introduction

Phytophthora foot rot, caused by *Phytophthora capsici*, is a serious threat to the production of black pepper (*Piper nigrum*) worldwide (Anandaraj, 2000). This oomycete is soil-borne, but also spreads through splashing of raindrops and infects roots, stem, leaves, and fruit at any stage of plant growth. Infection on plant parts below the ground cannot be detected during the initial stages of infection. Symptoms appear only at the advanced stages of root rot, and measures to control the disease at this juncture are futile. Preventive application of fungicides such as Bordeaux mixture, copper oxychloride, metalaxyl-mancozeb, or potassium phosphonate (Anandaraj and Sarma, 1995) to the soil or foliage is the recommended practice. A phenyl amide fungicide, namely metalaxyl, was found to be highly effective (Daggett *et al.*, 1993) but its high efficacy and specificity prompted its widespread use, which led to the emergence of *P. infestans* isolates resistant to the fungicide. Such resistance was first reported in the mid-1980s in *P. infestans* from the Netherlands and Ireland (Davidse *et al.*, 1981a, 1981b; Dowley and O'Sullivan, 1981; Gisi *et al.*, 1985), followed by resistance to metalaxyl in Korea (Choi *et al.*, 1992 and Kim *et al.*, 2003). To counter such resistance, mixtures of fungicides are preferred and currently used for managing the diseases caused by *Phytophthora*. Such mixtures help in three ways, by (1) widening the spectrum of activity; (2) exploiting synergy between fungicides, which increases the overall impact and also permits lower doses of individual fungicides; and (3) delaying the emergence of resistant isolates. Ramachandran (1990) studied the sensitivity of *P. capsici* isolates of black pepper to metalaxyl, and Veena *et al.* (2010) found a differential response in 29 isolates of *P. capsici* to potassium phosphonate in black pepper. Given the wide morphological diversity among *Phytophthora* strains isolated from different black-pepper-growing tracts as well as from different microclimates (the rhizosphere and different plant parts), it is imperative to study the isolates for variation in virulence and sensitivity to metalaxyl. The present work widened the scope of that

*Corresponding Author: arajiisr@gmail.com

objective by looking for any correlation between these attributes and the location from which the isolates had been collected and also between sensitivity to the fungicide and virulence.

Materials and methods

Phytophthora isolates

Eighty-two isolates of *Phytophthora,* representing all the three major states in India in which black pepper is grown, were chosen for the study: 44 from Kerala, 36 from Karnataka, and 2 from Tamil Nadu (Table 1). These isolates form part of the National Repository of *Phytophthora* maintained at the ICAR-Indian Institute of Spices Research (IISR), Kozhikode, Kerala. The isolates had been collected over time (1996–2010) from roots, stems, leaves, and fruits (berries) of black pepper and also from the collar region and the rhizosphere. The isolates maintained in sterile water were purified using PVPH medium (Tsao and Guy, 1977) and sub-cultured onto carrot agar (CA) when required.

Metalaxyl sensitivity

Sensitivity to the fungicide metalaxyl-mancozeb was determined by growing the isolates on CA supplemented with a commercially available formulation of the fungicide mixture (available as a wettable powder, containing eight per cent metalaxyl and 64 per cent mancozeb).

A preliminary study indicated that there was complete inhibition at 49.5 ppm and hence, further lower concentrations were used in this study. Stock solution of the fungicide (200 ppm) was prepared in sterile distilled water. Appropriate quantities of the stock solution were incorporated into sterilized CA, which was cooled to 49 °C before mixing, to obtain the following concentrations: 0.9, 4.5, 9.0, 13.5,18, 22.5, 27, 36, 45, and 49.5 ppm (0.1, 0.5,

Table 1. Origins and identity of *Phytophthora* isolates

Sl. No.	Isolate no.	Identity	Place of collection	Plant part / soil	District	Latitude (N)	Longitude (E)
1	96-08	*P. capsici*	Bhairumbe	Leaf	Sirsi	14°70′	74°83′
2	96-09	*P. capsici*	Bhairumbe	Leaf	Sirsi	14°70′	74°83′
3	97-11	*P. capsici*	Pulpally	Leaf	Wayanad	11°80′	76°17′
4	97-51	*P. capsici*	Pulpally	Leaf	Wayanad	11°80′	76º17′
5	97-55	*P. capsici*	Valparai	Root	Tamil Nadu	10°34′	76°95′
6	98-01	*P. palmivora*	Thamarassery	Soil	Kozhikode	11°77′	75°50′
7	98-03	*P. capsici*	Peruvannamuzhi	Soil	Kozhikode	11°60′	75°85′
8	98-17	*P. capsici*	Pulpally	Leaf	Wayanad	11°80′	76°17′
9	98-48	*P. capsici*	Madikeri	Leaf	Kodagu	12°43′	75°76′
10	98-59	*P. capsici*	Chettalli	Leaf	Kodagu	12°37′	75°84′
11	98-60	*P. capsici*	Valnoor	Leaf	Kodagu	12°40′	75°90′
12	98-70	*P. capsici*	Vageri	Leaf	Kodagu	12°30′	75°81′
13	98-75	*P. capsici*	Wayanad	Collar	Wayanad	11º41′	76º04′
14	98-76	*P. capsici*	Suntikoppa	Leaf	Kodagu	12°45′	75°89′
15	98-95	*P. nicotianae*	Adivaram	Stem	Wayanad	11°49′	76°02′
16	98-128	*P. capsici*	Kunnamangalam	Stem	Kozhikode	11°31′	75°87′
17	98-135	*P. capsici*	Theli	Root	Sirsi	14°62′	74°83′
18	98-143	*P. capsici*	Yadally	Root	Sirsi	14°62′	74°83′
19	98-149	*P. capsici*	Mukkal	Stem	Dharward	15°07′	75°01′
20	98-156	*P. capsici*	Sirsi	Soil	Sirsi	14°62′	74°83′

21	98-165	*P. capsici*	Balagadde	Root	Chikmagalur	13°38′	75°15′
22	98-171	*P. capsici*	Puttur	Leaf	Dakshina Kannada	12°76′	75°20′
23	98-172	*P. capsici*	Rayarpet	Leaf	Sirsi	14°62′	74°83′
24	98-177	*P. capsici*	Rayerpet	Leaf	Sirsi	14°36′	74°49′
25	98-183	*P. capsici*	Yadally	Leaf	Uttara Kannada	14°62′	74°83′
26	99-136	*P. capsici*	Wayanad	Leaf	Wayanad	11°41′	76°04′
27	99-139	*P. capsici*	Meenangadi	Leaf	Wayanad	11°66′	76°17′
28	99-144	*P. capsici*	Pollibetta	Leaf	Kodagu	12°32′	75°83′
29	99-145	*P. capsici*	Sugandhagiri	Spike	Wayanad	11°62′	76°06′
30	99-166	*P. capsici*	Peruvannamuzhi	Leaf	Kozhikode	11°60′	75°85′
31	00-38	*P. capsici*	Siddapur	Soil	Uttara Kannada	14°20′	74°55′
32	03-10	*P. capsici*	Thikkodi	Root	Kozhikode	11°49′	75°63′
33	05-03	*P. capsici*	Chelavoor	Leaf	Kozhikode	11°30′	75°84′
34	05-05	*P. capsici*	Peruvannamuzhi	Leaf	Kozhikode	11°60′	75°85′
35	05-09	*P. capsici*	Vaduvanchal	Collar	Wayanad	11°56′	76°22′
36	05-13	*P. capsici*	Appangala	Leaf	Kodagu	12°42′	77°35′
37	05-19	*P. capsici*	Chettalli	Soil	Kodagu	12°37′	75°84′
38	06-02	*P. capsici*	Koothali	Stem	Kozhikode	11°59′	75°76′
39	06-04	*P. capsici*	Puthupadi	Leaf	Kozhikode	11°48′	76°00′
40	06-08	*P. capsici*	Thiruvampadi	Leaf	Kozhikode	11°36′	76°02′
41	06-09	*P. capsici*	Iritti	Leaf	Kannur	11°98′	75°68′
42	06-11	*P. capsici*	Iritti	Berry	Kannur	11°98′	75°68′
43	06-17	*P. capsici*	Yercaud	Leaf	Salem	11°48′	78°13′
44	07-01	*P. capsici*	Peruvannamuzhi	Soil	Kozhikode	11°60′	75°85′
45	07-02	*P. capsici*	Peruvannamuzhi	Stem	Kozhikode	11°60′	75°85′
46	07-03	*P. capsici*	Chelavoor	Leaf	Kozhikode	11°30′	75°84′
47	07-07	*P. capsici*	Wayanad	Berry	Wayanad	11º41′	76º04′
48	08-01	*P. capsici*	Peruvannamuzhy	Root	Kozhikode	11°60′	75°85′
49	08-02	*P. capsici*	Thamaraserry	Leaf	Kozhikode	11°77′	75°50′
50	08-04	*P. nicotianae*	Chelavoor	Soil	Kozhikode	11°30′	75°84′
51	08-05	*P. capsici*	Koppa	Collar	Uttara Kannada	12°72′	76°96′
52	08-06	*P. capsici*	Peruvannamuzhy	Soil	Kozhikode	11°60′	75°85′
53	09-02	*P. capsici*	Peruvannamuzhy	Collar	Kozhikode	11°60′	75°85′
54	09-08	*P. capsici*	Coorg	Leaf	Kodagu	12°25′	74°45′
55	09-10	*P. capsici*	Kalpatta	Leaf	Wayanad	11°61′	76°08′
56	09-11	*P. capsici*	Peruvannamuzhi	Leaf	Kozhikode	11°60′	75°85′
57	09-12	*P. capsici*	Chelavoor	Berry	Kozhikode	11°30′	75°84′
58	09-13	*P. capsici*	Eriapally	Soil	Wayanad	11°80′	76°17′
59	09-14	*P. capsici*	Mananthavady	Berry	Wayanad	11°81′	76°00′
60	09-16	*P. capsici*	Padamala	Leaf	Wayanad	11°49′	76°04′

61	09-19	*P. capsici*	Therthalli	Soil	Kannur	12°21′	75°46′
62	09-20	*P. capsici*	Udayagiri	Soil	Kannur	12°24′	75°49′
63	09-21	*P. capsici*	Udayagiri	Soil	Kannur	12°24′	75°49′
64	09-22	*P. capsici*	Payamukku,	Soil	Kannur	11°98′	75°70′
65	09-25	*P. capsici*	Kanjirapuzha	Soil	Palakkad	10°99′	76°55′
66	09-27	*P. capsici*	Makkanduru	Soil	Coorg	12°46′	75°78′
67	09-28	*P. capsici*	Makkanduru	Soil	Coorg	12°46′	75°78′
68	09-33	*P. capsici*	Peruvannamuzhi	Root	Kozhikode	11°60′	75°85′
69	09-34	*P. capsici*	Udubanchola	Soil	Idukki	09°90′	77°20′
70	09-35	*P. capsici*	Peruvannamuzhi	Leaf	Kozhikode	11°60′	75°85′
71	09-36	*P. capsici*	Mudigere	Soil	Chikmagalur	13°12′	75°63′
72	09-37	*P. capsici*	Mudigere	Soil	Chikmagalur	13°12′	75°63′
73	09-39	*P. capsici*	Mudigere	Soil	Chikmagalur	13°12′	75°63′
74	09-40	*P. capsici*	Mudigere	Soil	Chikmagalur	13°12′	75°63′
75	09-41	*P. capsici*	Sakhleshpur	Soil	Hassan	12°95′	75°79′
76	09-42	*P. capsici*	Sakhleshpur	Soil	Hassan	12°95′	75°79′
77	10-01	*P. capsici*	Somvarpet	Soil	Hassan	12°59′	75°85′
78	10-02	*P. capsici*	Sakhleshpur	Leaf	Hassan	12°95′	75°79′
79	10-03	*P. capsici*	Madikeri	Soil	Kodagu	12°43′	75°76′
80	10-04	*P. capsici*	Madikeri	Leaf	Kodagu	12°43′	75°76′
81	10-05	*P. capsici*	Virajpet	Leaf	Kodagu	12°20′	75°80′
82	10-06	*P. capsici*	Virajpet	Soil	Kodagu	12°21′	75°80′

1.0, 1.5, 2.0, 2.5, 3, 4, 5, and 5.5 ppm of metalaxyl respectively) (Parra and Ristaino, 2001; Silvar *et al.*, 2006). Agar plugs (5 mm in diameter) cut from the growing margins of 72-hour-old cultures of the isolates were placed at the centre of the plates containing different concentrations of the fungicide and incubated at 25 °C for 72 h. Control plates (without the fungicide) were subjected to the same procedure. The experiment was laid out as a three-factor completely randomized design with three replicates, and each concentration was replicated three times. The colony diameter was measured at 72 h and percentage inhibition (PI) was calculated as follows:

PI = $[C – T/C]\times100$, where, C is the measure of growth of the pathogen in control plate (mm) and T is the measure of growth of the pathogen in treatments.

Based on the PI, the effective concentrations (EC_{50} and EC_{90}) were calculated by probit analysis using SPSS (ver. 19) and the isolates were scored as sensitive (S), intermediate (I), or insensitive/ less sensitive (L), depending on the concentration (ppm) of metalaxyl-mancozeb in CA *viz.,* S - EC_{90} at less than 10 ppm; I - EC_{90} at 10 to 40 ppm; L - EC_{90} at more than 40 ppm. Furthermore, values of EC_{50} for each isolate were calculated by plotting the percentage of growth of each isolate against log10 of the metalaxyl-mancozeb concentration.

Virulence assay

All the isolates were tested for their virulence on black pepper based on the detached leaf assay (Bhai *et al.*, 2010; Forbes, 1997). Leaves from three-month-old rooted cuttings of Subhakara, a variety of black pepper susceptible to *Phytophthora*, which were raised in a greenhouse were used for the assay. The second leaf from top was excised, washed thoroughly with sterile water, and blotted dry with a sterile blotting paper. Mycelial plugs, 5 mm in diameter and cut from the growing margins of 72-hour-old cultures of each isolate were placed on

the abaxial surface of the leaves and kept in closed plastic boxes lined with moistened filter paper. A small cotton pad moistened with sterile water was placed on the culture disc to keep the disc moist. The boxes were then incubated at 24±1 ºC. Lesions diameter was recorded every 24 h for 72 h and the diameter recorded at 72 h was used for scoring the virulence of the isolates on a scale of 1 to 4 as follows: 1 = no lesion (non-virulent), 2 = 1–10 mm (less virulent), 3 = 10.1–30 mm (moderately virulent), and 4 = more than 30 mm (highly virulent).

The data were analysed using the general linear model (GLM) procedure of SAS ver. 9.3 (SAS Institute, Cary, North Carolina) and the mean value comparisons were separated using Fischer's least significant difference (LSD) test. As some of the values were zero, square-root transformations (x + 0.5) were used for normalizing the variance. The entire experiment was repeated twice, and the data were pooled for statistical analysis. Significance was defined at p<0.05.

Results and discussion

Sensitivity to metalaxyl

The *in vitro* studies revealed that the isolates were highly variable in terms of their sensitivity to metalaxyl-mancozeb where the EC50 value ranged from 0.0001 ppm to 14.31 ppm. The lowest EC50 was shown by an isolate (Accession No. 98-156) from Sirsi, Karnataka. Values of EC90 ranged from 0.49 ppm to 68.50 ppm. Among the 82 isolates, 19 (23.2%) were highly sensitive (EC90 0.49–9.88 ppm). Six isolates (7.3%) were the least sensitive (EC90 41.31–68.50 ppm at concentrations below 40 ppm) (Table 2, Fig. 1 and Fig. 2). A majority of the isolates (69.5%) were in the intermediate range. However, these sensitive isolates showed no correlation between sensitivity and location of isolation.

Although only a few isolates proved resistant to metalaxyl-mancozeb in the present experiment as there is no wide spread use of metalaxyl in India for black pepper. Whereas, in the tropical highlands of Africa where potatoes are grown, it was reported that 86 per cent of *P. infestans* isolates from Kenya and 59 per cent from Uganda were resistant (Erselius *et al.,* 1999). In Uganda, 44 per cent of *P. infestans* isolates were reported to be resistant to

Table 2. Metalaxyl sensitivity *in vitro* amongst *Phytophthora* isolates of black pepper

Sl. No.	Isolate no.	EC50	EC90	Reaction
1	98-156	0.00	0.49	S*
2	10-03	0.01	0.55	S
3	06-04	0.00	1.13	S
4	09-10	0.02	1.92	S
5	09-21	0.00	3.42	S
6	05-03	2.34	3.98	S
7	05-13	1.44	4.81	S
8	03-10	2.02	5.60	S
9	06-09	1.67	5.75	S
10	09-22	0.01	6.11	S
11	08-01	2.46	7.21	S
12	09-16	0.01	7.27	S
13	10-06	0.87	7.56	S
14	06-11	2.64	7.98	S
15	09-13	0.11	8.48	S
16	10-01	1.98	8.66	S
17	09-28	0.89	8.74	S
18	09-40	0.39	8.84	S
19	05-05	4.55	9.88	S
20	08-04	5.60	10.22	I*
21	08-06	2.92	10.35	I
22	10-04	3.75	10.99	I
23	99-166	1.24	11.35	I
24	05-09	3.97	11.76	I
25	09-37	1.21	11.77	I
26	98-128	8.03	12.14	I
27	09-12	4.94	12.49	I
28	09-02	4.66	12.76	I
29	98-135	1.23	12.85	I
30	06-17	0.91	12.94	I
31	09-42	1.77	13.28	I
32	09-25	2.25	13.68	I
33	98-172	5.57	14.19	I
34	06-02	5.70	14.19	I
35	98-60	9.42	14.21	I
36	98-01	9.61	14.50	I
37	96-08	10.84	15.01	I
38	09-08	3.81	15.29	I
39	09-36	2.06	16.08	I
40	09-27	4.49	16.15	I
41	99-139	8.38	16.24	I

42	05-19	9.13	16.55	I
43	98-149	12.44	16.92	I
44	09-41	6.30	17.12	I
45	97-55	3.23	17.36	I
46	09-39	6.39	17.49	I
47	98-95	8.71	17.80	I
48	07-03	2.76	17.88	I
49	98-03	4.29	17.92	I
50	09-34	3.92	18.08	I
51	98-59	4.85	18.34	I
52	09-11	7.77	18.65	I
53	08-05	2.22	18.89	I
54	09-19	1.94	19.15	I
55	98-70	14.96	19.22	I
56	99-145	14.60	19.38	I
57	09-20	1.66	19.98	I
58	06-08	2.87	20.47	I
59	96-09	11.37	20.55	I
60	99-144	8.51	21.09	I
61	98-17	21.68	22.03	I
62	00-38	21.75	22.10	I
63	09-33	2.32	22.12	I
64	97-11	15.87	22.14	I
65	98-76	8.73	22.47	I
66	99-136	21.89	23.23	I
67	98-143	13.42	23.50	I
68	09-35	13.21	24.32	I
69	07-07	4.06	24.96	I
70	98-165	12.33	27.64	I
71	97-52	6.42	28.06	I
72	98-171	10.55	28.44	I
73	09-14	4.87	28.58	I
74	10-02	13.05	34.64	I
75	98-183	5.52	37.91	I
76	98-48	10.22	39.75	I
77	08-02	1.89	41.31	L*
78	98-177	8.14	42.37	L
79	07-01	14.45	45.86	L
80	98-75	8.42	51.73	L
81	10-05	18.21	52.05	L
82	07-02	14.31	68.50	L

***S** = sensitive-23.2%; **I** = intermediate-69.5%; **L**= less sensitive-7.3%

metalaxyl-mancozeb and 24 per cent were moderately resistant (Mukalazi *et al.,* 2001). High frequencies of metalaxyl resistance in *P. infestans* have also been reported elsewhere (Cohen and Coffey, 1986; Davidse *et al.,* 1981b; Deahl *et al.,* 1993; Gisiand Cohen, 1996; McLeod *et al.,* 2001; Vega-Sanchez *et al.,* 2000). Thind *et al.* (2001) reported variation in sensitivity to metalaxyl in *P. infestans* in Punjab, India. Out of the 68 populations studied, three were identified as resistant to metalaxyl. Fontem *et al.* (2005) studied the pathogenicity and metalaxyl sensitivity of *P. infestans* isolates obtained from garden huckleberry, potato, and tomato in Cameroon and found that the aggressiveness of the isolates increased with their resistance to metalaxyl. The sensitivity of these isolates to metalaxyl varied with

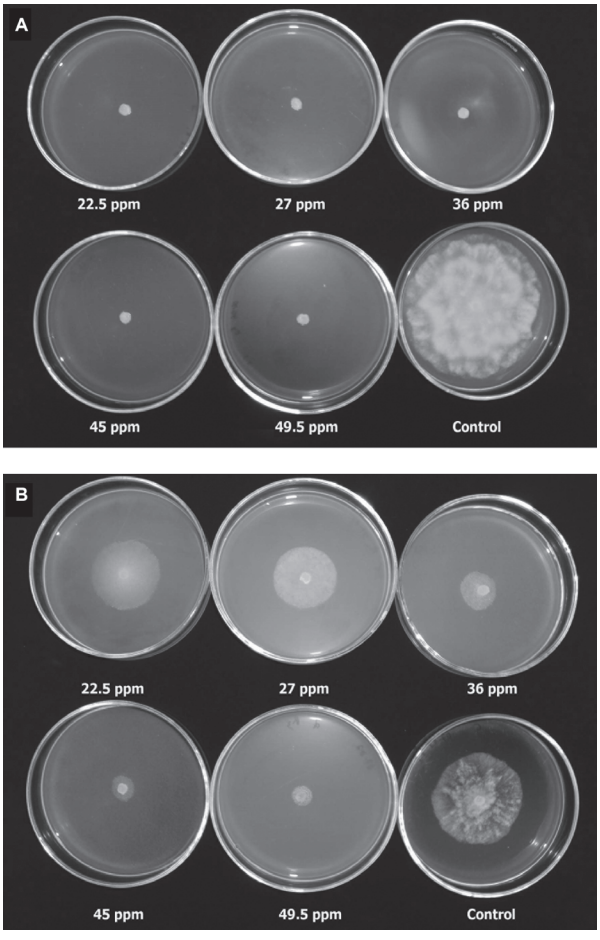

Fig. 1. Sensitivity assay of *Phytophthora* isolates of black pepper to metalaxyl-mancozeb: Isolate no. 98-156, highly sensitive (A), Isolate no. 07-02, least sensitive (B)

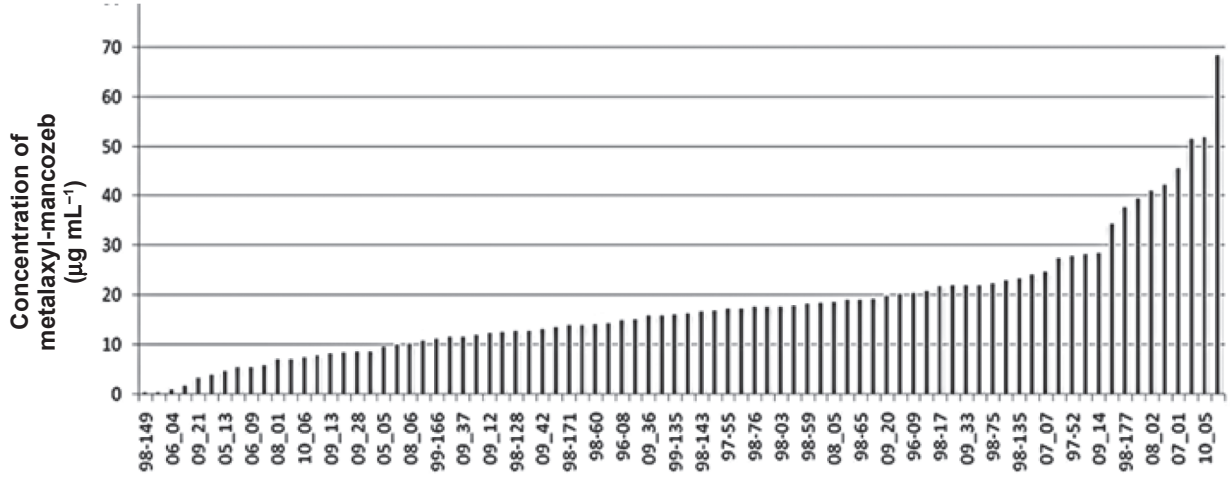

Fig. 2. Sensitivity of *Phytophthora* isolates of black pepper to metalaxyl-mancozeb as a function of its concentration

the location from which they had been collected and with the source (primary hosts), with isolates from huckleberry showing the lowest proportion (34%) of metalaxyl-resistant isolates. Among the 233 isolates tested for metalaxyl sensitivity, 49 per cent were resistant to metalaxyl in 2001 and 51 per cent in 2002. Dunn *et al.* (2010) studied the population structure and resistance to mefenoxam of *P. capsici* in New York State and found that the population to be highly diverse, but the gene flow among regions and fields to be restricted. Therefore, they concluded that the population from each field needs to be considered as independent population, and the movement of inoculum across the fields restricted to prevent the spread of the pathogen. Overall, worldwide populations of *P. capsici* vary in their sensitivity to fungicides and in virulence. Although only very few of the *Phytophthora* isolates of black pepper were resistant to metalaxyl, it is important to consider segregation as one of the disease management strategies. Our results are also very well supported by the work of Silvar *et al.* (2006), who characterized *P. capsici* isolates obtained from pepper (*Capsicum annuum* L.) in North-western Spain based on virulence, mating type, sensitivity to metalaxyl, and genetic analysis using random amplified polymorphic DNA methods and found no correlation between the categorization based on RAPD analysis and that based on virulence or response to metalaxyl. Davidse *et al.* (1983) reported RNA polymerase as the target site for metalaxyl, and any alteration in the target site can lead to resistance in some pathogenic oomycetes. From the genetic studies of *P. infestans* and *P. sojae,* Bhat *et al.* (1993) and Lee *et al.* (1999) reported that resistance was linked with a single dominant gene, and variations in resistance were accounted for by the influence of minor genes. Fabritius (1997) believed that metalaxyl resistance in *P. infestans* developed independently in different areas of the world.

Virulence assay

Among the total isolates, only five (6.1%) were non-virulent (they failed to induce any lesion); four (3.7%) proved to be less virulent (lesion diameter 2.6–3.6 mm); and the rest were either moderately virulent (47.6%) or highly virulent (41.5%; lesion

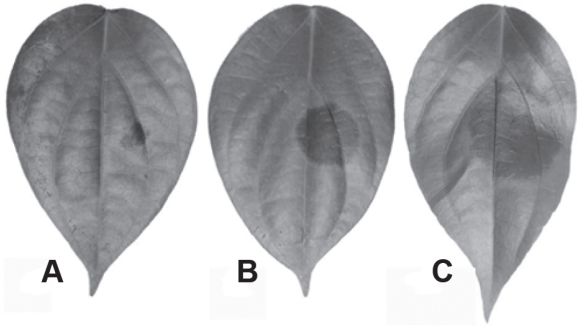

Fig. 3. Variation in virulence of *Phytophthora* isolates of black pepper: (A) Less virulent, (B) Moderately virulent and (C) Highly virulent

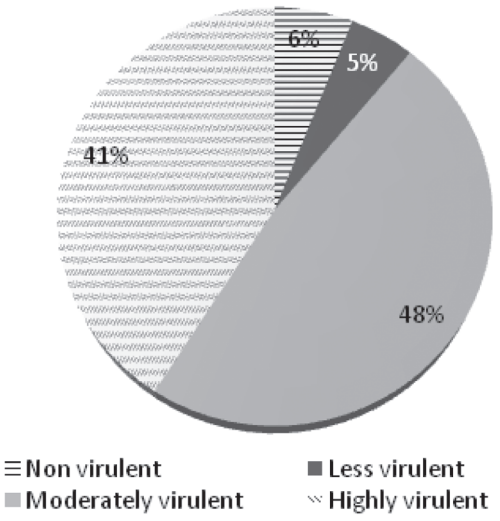

Fig. 4. Virulence of *Phytophthora* isolates of black pepper

diameter 17.0–28.3 mm) (Fig. 3). All the highly virulent isolates, 69.2% of the moderately virulent isolates, and only one among the less virulent isolates produced signs of infection on the leaves within 24 h of inoculation whereas the rest took 48 h or more to do so (data not shown).

Though the isolates varied in the degree of aggressiveness in infecting the susceptible black pepper variety, neither virulence nor sensitivity to metalaxyl-mancozeb could be correlated with the location (origin) from which the isolates had been collected. Among the 82 isolates, only five were non-virulent but the age of the culture had nothing to do with the degree of virulence. The isolates collected in 1997 (97-52) were highly virulent whereas those collected in 2009 (09-25) were non-virulent. Hwang *et al.* (1996) reported similar results in their studies on the variation in virulence in naturally occurring isolates of *P. capsici*. Variation in virulence among isolates of *P. cactorum* and *P. parasitica* was reported by Hantula*et al.* (1997), Lebretonand Adrivon (1998), and Matheron and Matejka (1990). Similarly, varying levels of disease were observed with nine isolates of *P. capsici* on nine Korean and Japanese pumpkin cultivars (Lee *et al.,* 2001), thus showing the difference in virulence among isolates. The diversity in virulence observed among *P. capsici* isolates of black pepper in this study is well supported by these studies. The sensitivity to metalaxyl-mancozeb and virulence were independent attributes. Out of 82 isolates studied, 41.5 per cent of the isolates were highly virulent but only 7.3 per cent were resistant to metalaxyl-mancozeb.

Conclusions

Only a small proportion (7.3%) of the isolates was resistant to metalaxyl-mancozeb, suggesting that the chance of developing resistance by the pathogen for this combination is negligible. Unlike vegetables in general and potato in particular the use of metalaxyl to manage *Phytophthora* infection is very negligible. That could be one of the reasons for the low frequency of resistant isolates encountered among black pepper isolates. The highly sensitive isolate from Sirsi 98-156 is from soil and never exposed to metalaxyl fungicide also suggests this. The farmers in this location manage *Phytophthora* by use of copper fungicides only as aerial spray. The variable sensitivity must be the intrinsic character of each isolate and not due to their exposure to the fungicide. There was no significant correlation between metalaxyl sensitivity and virulence of the isolates. Further studies are warranted to determine the molecular basis of this variation in fungicide sensitivity. Such studies can also help to identify markers linked to virulence/avirulence loci in *Phytophthora* of black pepper.

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