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PLANT PROTECTION
Article

Distributional spectrum of bacterial wilt of chili incited by *Ralstonia solanacearum* in Pakistan

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ABSTRACT: The production of chili is seriously threatened by bacterial wilt incited by *Ralstonia solanacearum* in Pakistan. As the information about the distribution and prevalence of the disease and the pathogen is scarce, the present study was performed to ascertain the prevalence, occurrence and distribution of *R. solanacearum* in different regions of chili cultivation. The results showed differences in the incidence and prevalence of bacterial wilt all over the country. Overall, an incidence of 10% and prevalence of 76% of the disease was observed in the country. The highest incidence of bacterial wilt was observed in the province of Sindh whereas it was the lowest in Baluchistan. The prevalence of the disease followed the similar trend in the provinces. As regards agroecological zones, the highest incidence was recorded in Indus delta followed by sandy deserts whereas it was the lowest in western dry mountains. Almost the same tendency was observed regarding prevalence in the eight agroecological zones. In case of districts, the disease incidence was the maximum in Badin while it was the minimum in the district of Loralai. On the other hand, the disease prevalence was the maximum (100%) in the districts of Badin, Mirpurkhas, and Thatta and the minimum (60%) in the districts of Barkhan, Karak, and Loralai. Of the total 114 isolates of *R. solanacearum*, 81% were confirmed as Biovar 3 whereas the rest 19% isolates were identified as Biovar 4. The findings will help the farmers to design disease management programs accordingly to avert yield losses.

Key words: vigilance, *Capsicum annuum*, biovar, distributional differences, incidence.

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum* is a serious threat to solanaceous crops both in temperate and tropical regions of the world (EPPO 2020; Hayward 1991). Bacterial wilt is ubiquitous in distribution with varying proportions. In Bangladesh, up to 31% disease incidence has been reported on eggplant. An incidence of 55% and 25% has been recorded on chili and potato crops respectively from the major chili and potato producing regions of Ethiopia (Bekele et al. 2011). In Peru, in the Amazon basin, banana plantations were found affected with *R. solanacearum* and had to be demolished due to quick spread of the pathogen all over the Peruvian Jungle (French and Sequeira 1968). The pathogen has been reported to invade over 450 plant species from 54 botanical families and incur huge yield losses (Denny 2006; Genin and Denny 2012; Wicker et al. 2007). As more hosts are being identified each year, the bacterium's list of host plants is growing (He, Z. et al. 2008; Liu et al. 2009; She et al. 2012; 2013; Wang et al. 2009; Xu et al. 2009). The maximum damages were reported on potato, tomato and tobacco in USA, Brazil, Columbia, South Africa and Indonesia. In Philippines, 15% average losses were recorded on tomato crop, 10% in capsicum and aubergine, and 2–5% in tobacco (Zehr 1969). Losses of 30–70% in potato and up to 65% in brinjal in India and 30% in peanut in China have been reported (Sitaramaiah and Sinha 1984). In India, the bacterium caused complete failure of tomato crop. Widespread losses on potato have also been reported in Greece (Nisa et al. 2022). The bacterium has also been reported to be implicated in disease complexes. The synergistic

interactions between *R. solanacearum* and root-knot nematodes resulted in heavy losses as compared to their individual losses, rendering the plants prone to bacterial wilt (Asghar et al. 2020; Furusawa et al. 2019; Getu et al. 2021; Ghosh et al. 2016; Junaid et al. 2020; Khan and Siddiqui 2017; Shahid et al. 2022).

Bacterial wilt has also been reported from Pakistan infecting many host plants. It was first reported from Pakistan in 1989 (Aslam et al. 2017a, b; 2019; Geddes 1989). The disease is a major production constraint for solanaceous crops including chili. Pakistan is among the major chili producing countries, ranking 5th in cultivation and 10th in production in the world (FAO 2012). The yield of chili obtained in Pakistan is quite low (2.53 tons·h^{-1}) as compared to Morocco (22.04 tons·h^{-1}) and many other developed countries which can be ascribed to a plethora of biotic factors; *R. solanacearum* being among the major constraints. The pathogen has been categorized into five biovars and five races in different regions of the world and its management is difficult due to its diversified and complex nature (Buddenhagen et al. 1962; Hayward 1964; 1991; 1994; He, L. et al. 1983; Kelman et al. 1994; Pegg and Moffett 1971).

In Pakistan, little work has been done on this pathogen (Burney 1995) and the information regarding its incidence and prevalence particularly on chili crop in different agroecological zones of Pakistan with different climatic conditions and edaphic factors is exiguous. Ergo, the objective of the current study was to determine the incidence, prevalence and biovar distribution of *R. solanacearum* in different agroecological zones. The study will help the farmers in designing control strategies against the bacterium accordingly.

MATERIALS AND METHODS

Description of the studied areas

The studies on the determination of incidence and prevalence of *R. solanacearum* inciting bacterial wilt of chilies and distribution of biovars of *R. solanacearum* were conducted in eight agroecological zones of Pakistan. Pakistan is situated between latitude 30°00' N and longitude 70°00' E in Asian subcontinent. The climate of Pakistan is almost dry and intense, extremely hot in summers and extremely cold in winters with less rainfall and varies from place to place. The northern parts are having high mountains intermingled with valleys and, in the southwards, there is the Pothowar region followed by Indus plain, 322 km wide and 1,287 km long with 1% inclination from north to south. The Baluchistan plateau is in the western parts bordering low to high mountains from north to east. The country has two sandy deserts in the Indus basin; the Thar Desert in the lower part and the Thal Desert in the upper part. With diversified ecologies, Pakistan has been classified into different agroecological zones. The salient features of these agroecological zones of the country have been described in Tables 1 and 2.

Table 1. Meteorological parameters of eight agroecological zones of Pakistan.

Sr. No	Agroecological zone	Districts	Temperature (˚C)		Rain fall (mm/month)		Relative humidity (%)
			Summer	Winter	Summer	Winter	
1	Indus Delta	Thatta, Badin	34-45	19-20	75	> 5	67-87
2	Southern Irrigated Plain	Mir Pur Khas, Umer Kot	40-45	8-12	16-20	> 4	55-60
3	Sandy desert	Bahawalpur, Sanghar	39-41	7-10	32-46	> 4	44
4	Northern Irrigated Plain	Multan, Pakpattan, Kasur	41-48	6-28	75-108	14-22	51
5	Barani areas	Attock	35-38	3-6	200	36-50	56
6	Wet Mountains	Nowshera	35	0-4	236	116	64
7	Western Dry Mountains	Karak, Loralai	30-39	-3-7.7	45-95	47	58
8	Sulaiman Piedmont	Barkhan	40.5	5.7-7.6	21-38	13	32

Table 2. Soil conditions and major crops of eight agroecological zones of Pakistan.

Sr. No.	Agroecological zone	Districts	Soil type	Soil pH	Organic matter (%)	Major crops
1	Indus Delta	Thatta, Badin	Clayey and silty	7.6-8.2	0.5-0.8	cotton, sugarcane, wheat, maize, millet, barley and vegetables
2	Southern Irrigated Plain	Mirpur Khas, Umer Kot	Silty and sandy loam	6.8-7.2	0.4-0.5	cotton, sugarcane, wheat, maize, millet, barley and vegetables
3	Sandy desert	Bahawalpur, Sanghar	Sandy soils and moving dunes with strips of clayey soils	7.8-8.3	0.4	cotton, sugarcane, wheat, maize, millet, barley, xerophytic vegetation and vegetables
4	Northern Irrigated Plain	Pakpattan, Multan, Kasur	Sandy loam, clay loam, silt loam and 15 % saline-sodic	7.5-7.8	04-0.6	cotton, sugarcane, wheat, oilseeds, rice
5	Barani areas	Attock	Non-calcareous to moderately calcareous, silt loams and with west southern part mainly calcareous	7.5-7.7	< 0.5	sorghum, millet, maize, pulses, groundnut, wheat and vegetables
6	Wet Mountains	Nowshera	Silt loam to silty clays, non-calcareous to slightly calcareous	7.5-8.1	0.4-0.6	maize, wheat, fruits (apples), olives forests and vegetables
7	Western Dry Mountains	Karak, Loralai	Loamy, deep and calcareous	8.3	0.3-0.5	wheat, grazing, apples, plums, apricots, grapes, peaches and vegetables
8	Sulaiman Piedmont	Barkhan	Silt loam, deep and strongly calcareous	8.5	0.3-0.4	xerophytic vegetation, grasses, wild olives, fruits, vegetable and wheat

Distribution of *R. solanacearum*

For recording incidence of bacterial wilt in Pakistan, an extensive survey of chili was conducted in 14 major chili cultivating districts falling under eight agroecological zones of Pakistan (Fig. 1). From each district, 10 sites were randomly selected making a total of 140 sites. From each site one field of chili (~ 1 acre) was randomly selected and 50 chili plants were observed randomly following zigzag pattern for recording incidence of bacterial wilt. Wilted plants showing characteristic symptoms were recorded and the association of bacterium was confirmed by immunostrips in the field (Opina and Miller 2005). The incidence of bacterial wilt of each site was calculated as described by Fateh et al. (2022). Similarly, the incidences from all the districts, agroecological zones, provinces and finally the whole country were calculated. Disease prevalence of bacterial wilt in each district, agroecological zone, province and the country was also calculated as described by Fateh et al. (2022).

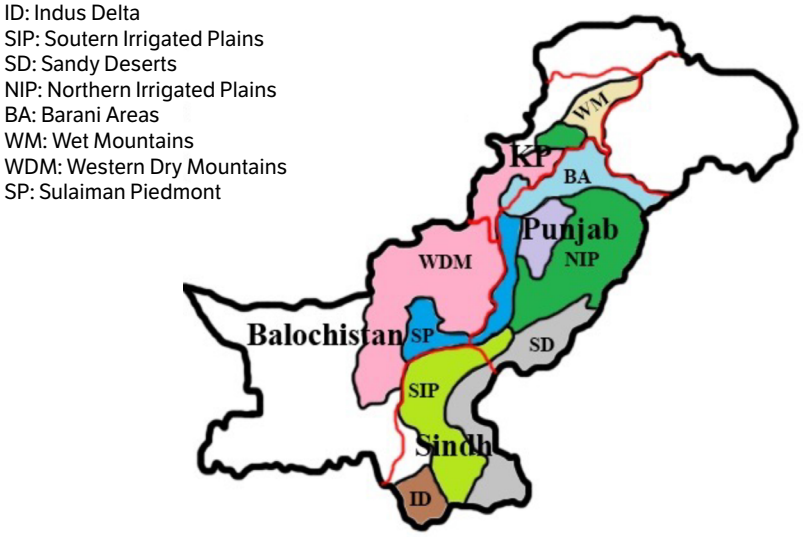

Figure 1. Map showing agroecological zones of Pakistan.

Symptomatological confirmation of bacterial wilt

The wilted plants were identified by the characteristic symptoms of the disease. These symptoms included wilting, stunting and yellowing of foliage, leaf epinasty, adventitious root growth on the stem, narrow dark stripes corresponding to the infected vascular bundles beneath the epidermis. Internal symptoms included progressive discoloration of vascular tissues mainly xylem and appearance of slimy viscous ooze when the stems were cut transversely.

Serological confirmation of *R. solanacearum* in wilted plants

The association of *R. solanacearum* with the wilted plants in the field was confirmed serologically by using immunostrips (Opina and Miller 2005).

Collection of *R. solanacearum* isolates

A total of 114 isolates of *R. solanacearum* associated with chili were collected from 14 major chili growing districts falling under eight agroecological zones situated in four provinces of the country. Chili plants showing the characteristic symptoms of bacterial wilt were excavated carefully along with soil from the rhizosphere, placed in polythene bags, labeled (with host information, locality and date of collection), kept in cold place and brought to the laboratory for further analyses.

Isolation of *R. solanacearum*

The bacterium was isolated from soil and stem samples collected from different sites of each district of eight agroecological zones as described below.

Isolation from soil

Bacteria were isolated from soil by using serial dilution method. For this purpose, 1 g of soil was taken and homogenized in 9 mL of distilled water and dilution series of 10^6 and 10^7 were made by adding requisite amount of distilled water. By using micropipette, 100 µL from each dilution series of 10^6 and 10^7 were taken and spread on the Semi-selective Medium, South Africa (SMSA) media plates and incubated at 28 °C for 48 h for bacterial growth (Engelbrecht 1994).

Isolation from stem

Stem segments of approximately 10 cm in length of wilted plants were taken from collar region, surface sterilized with 70% ethanol and cut into small pieces. These pieces were then kept in 5 ml sterile distilled water for 5 min with continuous shaking in a shaker at room temperature. The bacterial suspension (100 µL) from each sample was streaked separately on the triphenyltetrazolium chloride (TTC) medium, spread uniformly and incubated as mentioned above (Hugh and Leifson 1953).

Purification of *R. solanacearum*

To obtain pure cultures, a single colony from each bacterial culture isolated from soil and stem were re-streaked on TTC and nutrient agar media under sterile conditions. The single colonies were taken again from TTC medium and re-streaked on SMSA media containing TTC, cycloheximide, bacitracin, and penicillin to avoid contamination.

Confirmation of *R. solanacearum* isolates

The purified cultures of 114 isolates of *R. solanacearum* were further confirmed serologically (Opina and Miller 2005) and by their hypersensitivity response.

Hypersensitive reaction

The isolates confirmed serologically were tested for hypersensitivity reaction on tobacco. Bacterial suspension of 10^8 CFU/mL from each isolate was prepared in sterilized distilled water and infiltrated into leaf mesophyll of tobacco plants by using sterilized syringe. The distilled water was used as a positive control. Each isolate was inoculated twice in the same leaf and the same procedure was repeated on three plants. The plants were incubated at 28 °C and observed after 24 and 48 h for the development of necrosis in the inoculated areas of the leaves. The confirmed purified isolates were coded accordingly.

Characterization of *R. solanacearum*

The isolates were characterized morphologically by their growth patterns (mucoid and nonmucoid growth) and biochemically by employing various biochemical tests (Atiq et al. 2022; Khurshid et al. 2022), viz. gram reaction, catalase activity, levan production (Rahoo et al. 2022; Schaad 1988), KOH loop test (Suslow et al. 1982), oxidase activity (Kovacs 1956), lipase activity, pigment production (King et al. 1954), arginine dihydrolase reaction (Thornley 1960), gas production (van den Mooter 1987), oxidation and fermentation activity (Hayward 1964).

Molecular confirmation

The DNA from the 114 purified isolates was extracted, quantified and amplified by using the primer pair JHFegl: 5'GACGATGCATGCCGCTGGTCGC 3' and JHRegl: 5' CACGAACACCACGTTGCTCGCATTGG 3'. Each polymerase chain reaction (PCR) amplification contained 1 unit of Taq DNA polymerase (GoTaq Flexi DNA Polymerase; Promega Corp., Madison, WI) with 5.0 µL of 5× buffer, 1.5 µL (25 µmol·L^{-1}) of MgCl2, 1.0 µL (10 µmol·L^{-1}) of each dNTP, each primer at 10 pmol, and 100 ng of DNA. The total volume was adjusted to 25 µL with sterile deionized water. A hot start of 95 °C for 5 min; followed by 30 cycles of 95 °C for 45 s, 68 °C for 30 s, and 72 °C for 60 s; and a 10-min extension at 72 °C in the last cycle was used for the amplification of a DNA sequence in a thermocycler (Bio-Rad, Hercules, CA). The annealing temperature was adjusted according to the composition of the oligonucleotide sequence. The PCR products electrophoresed through a 1% agarose gel were visualized with ultraviolet light after ethidium bromide staining (Anwar et al. 2022; Ashraf et al. 2022). All the isolates yielded a 750-bp band that corresponded to *R. solanacearum*.

Identification of biovars

The bacterial isolates were identified into biovars on the basis of utilization of different sugars. One gram of each disaccharides (maltose, cellobiose, lactose) and hexose alcohol (dulcitol, mannitol, sorbitol) was mixed with 9 mL of sterilized distilled water to make 10% of the solutions. The sugars were sterilized by filtering through 0.2 µm pore size filters (orange scientific, GyroDisc CA-PC sterile, endotoxin-free, hydrophilic with catalogue No. 1520012 having cellulose acetate membrane 30 mm) and from each sugar and carbohydrate, 10 mL was added in 190 mL of Ayer's medium, distilled water serving as control. The medium containing agar was plated, a suspension of bacterial culture at 10^8 CFU/mL was prepared and 25 µL was taken and inoculated onto the surface of Ayer's mineral base medium amended with carbohydrates. The plates were incubated at 28 °C and observed for the absence or presence of bacterial growth (Hayward 1964; He, L. et al. 1983).

RESULTS

Variations in incidence and prevalence of bacterial wilt

Variations in incidence and prevalence of bacterial wilt caused by *R. solanacearum* were recorded throughout the country. The overall incidence of 10% and prevalence of 76% of *R. solanacearum* was recorded in the country.

Of the four provinces, maximum disease incidence of 16.4% was recorded in the province of Sindh followed by Punjab and Khyber Pakhtunkhwa showing 11.4% and 7.0% disease incidences respectively. On the other hand, minimum incidence of 4.9% was observed in the province of Baluchistan. As regards prevalence, the same pattern was observed. The prevalence of bacterial wilt was the maximum (94.0%) in the province of Sindh followed by Punjab and Khyber Pakhtunkhwa, showing 84.0% and 65.0% disease prevalence. On the contrary, the minimum disease prevalence of 60.0% was observed in Baluchistan province as shown in Fig. 2.

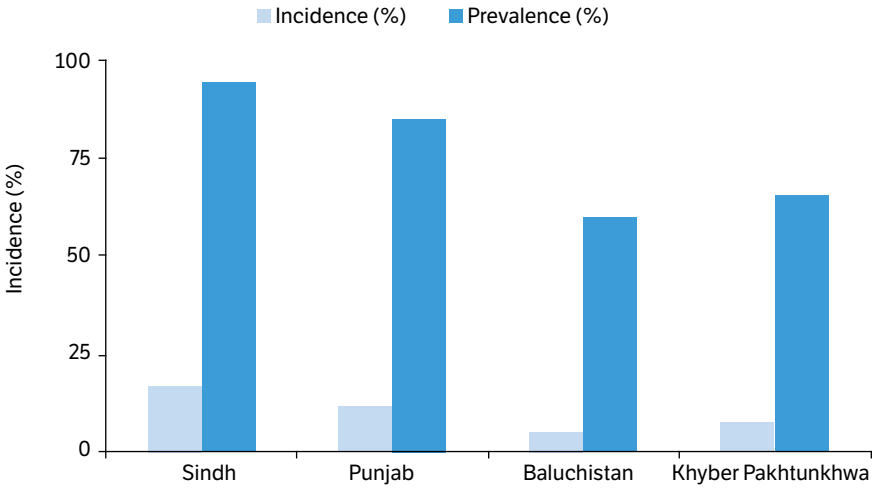

Figure 2. Incidence and prevalence of bacterial wilt in four provinces of Pakistan.

Out of eight agroecological zones, the maximum disease incidence of 19.5% was observed in Indus delta followed by Sandy deserts (14.1%) while the minimum disease incidence of 5.0% was found in western dry mountains. The disease incidence in other zones ranged between 5.4 and 14.1% as shown in Fig. 3. In case of disease prevalence, the maximum disease prevalence was observed in Indus delta which was found 100% followed by 90.0% of southern irrigated plains while the minimum disease prevalence of 70.0% was recorded in western dry mountains and the Suleiman piedmont. The prevalence ranged between 70.0 and 86.6% in other agroecological zones.

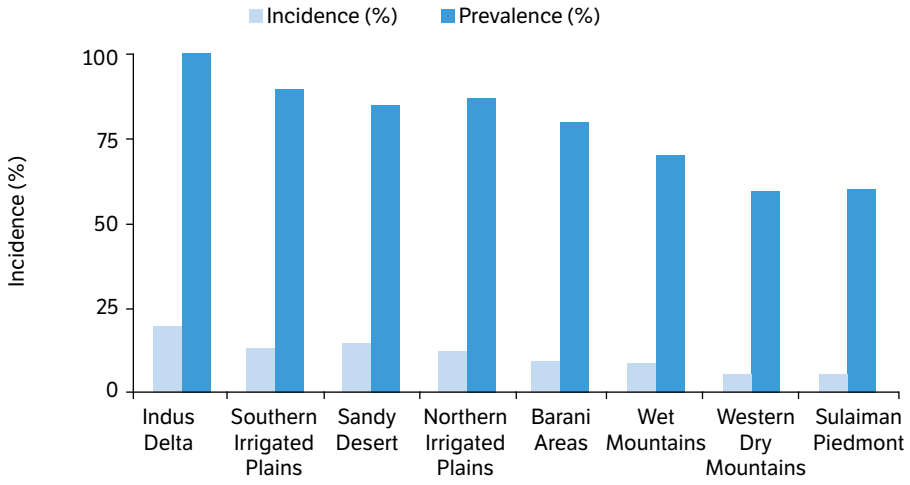

Figure 3. Incidence and prevalence of bacterial wilt in eight agroecological zones of Pakistan.

Of the 14 districts, the maximum disease incidence of 22% was observed in district Badin followed by district of Thatta (17%) while the minimum disease incidence of 4.4% was observed in the district of Loralai. The disease incidence was found variable in the remaining districts as shown in Fig. 4. Similarly, the maximum disease prevalence was found in the districts of Thatta, Badin and Mirpurkhas which were 100%, while the minimum disease prevalence of 60% was observed in Karak, Loralai and Barkhan. The rest of the districts had disease prevalence ranging from 70 to 90% (Fig. 5).

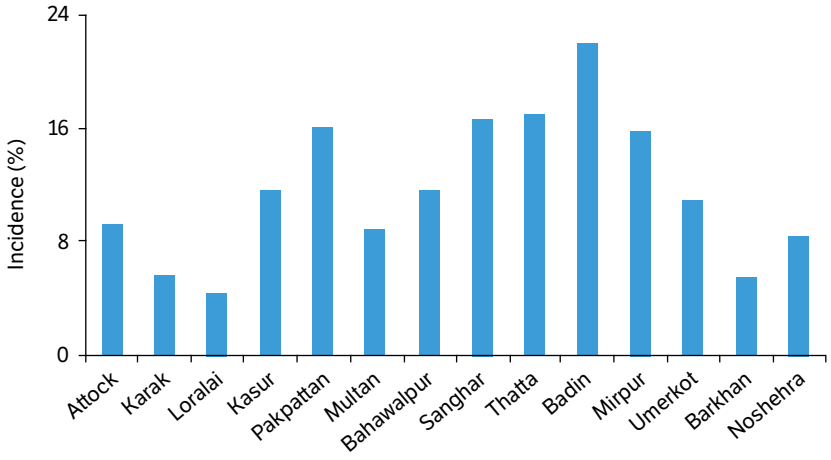

Figure 4. Incidence of bacterial wilt in 14 districts of Pakistan.

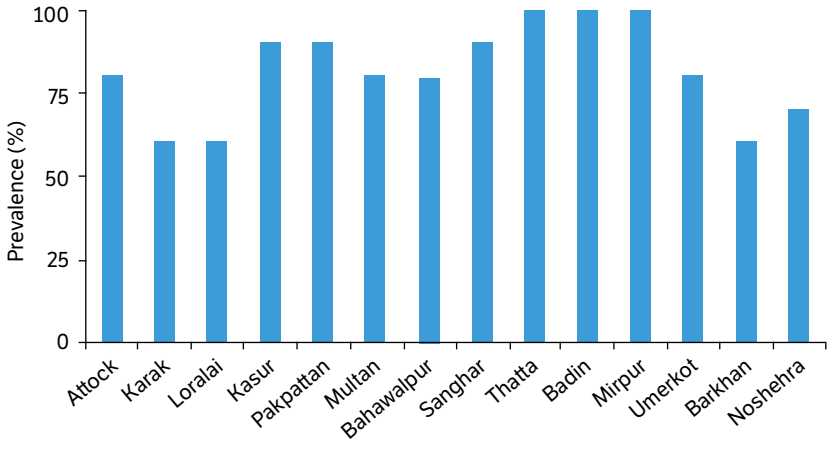

Figure 5. Prevalence of bacterial wilt in 14 districts of Pakistan.

Distribution of biovars

Out of 114 *R. solanacearum* isolates, 92 (81%) were identified as biovar 3, while the remaining 22 (19%) were recognized as biovar 4. Biovar 3 was recorded from all the four provinces and was found to be predominant in all the provinces while biovar 4 was found in the provinces of Punjab and Sindh only (Table 3). Similarly, biovar 3 was observed from all the eight agroecological zones and found to be predominant. On the other hand, biovar 4 was recorded from four agroecological zones. The zone wise and district wise distribution of biovars 3 and 4 is given in Table 4.

Table 3. Distribution of biovars of *R. solanacearum* in the four provinces of Pakistan.

Province	No. of isolates	%	Biovar 3	%	Biovar 4	%
Sindh	47	41	33	70	14	30
Punjab	42	37	34	81	08	19
Khyber Pakhtoonkhwa	13	11	13	100	-	-
Baluchistan	12	11	12	100	-	-
Pakistan	114	-	92	81	22	19

Table 4. Distribution of biovars of *R. solanacearum* in the eight agroecological zones and 14 districts of Pakistan.

Agroecological zone	District	No. of isolates	%	Biovar 3	%	Biovar 4	%
Indus delta	Thatta	10	8.8	6	60.0	4	40.0
	Badin	10	8.8	7	70.0	3	30.0
	Total	**20**	**17.5**	**13**	**65.0**	**7**	**35.0**
Southern irrigated plains	Mir Pur Khas	10	8.8	08	80.0	2	20.0
	Umer Kot	8	7.0	05	62.5	3	37.5
	Total	**18**	**15.8**	**13**	**72.2**	**5**	**27.8**
Sandy deserts	Bahawalpur	8	7.0	7	87.5	1	12.5
	Sanghar	9	7.9	7	77.8	2	22.2
	Total	**17**	**14.9**	**14**	**82.0**	**3**	**18.0**
Northern irrigated plains	Multan	8	7.0	6	75.0	2	25.0
	Pakpattan	9	7.9	6	66.7	3	33.3
	Kasur	9	7.9	7	77.8	2	22.2
	Total	**26**	**22.8**	**19**	**73.0**	**7**	**27.0**
Barani areas	Attock	8	7.0	8	100.0	-	-
	Total	**8**	**7.0**	**8**	**100.0**	**-**	**-**
Wet mountains	Nowshera	7	6.1	7	100.0	-	-
	Total	**7**	**6.1**	**7**	**100.0**	**-**	**-**
Western dry mountains	Karak	6	5.3	6	100.0	-	-
	Loralai	6	5.3	6	100.0	-	-
	Total	**12**	**10.5**	**12**	**100.0**	**-**	**-**
Suleiman piedmont	Barkhan	6	5.3	06	100.0	-	-
	Total	**6**	**5.3**	**06**	**100.0**	**-**	**-**
Pakistan	**Grand total**	**114**	**-**	**92**			

DISCUSSION

Ralstonia solanacearum, the causal organism of the bacterial wilt, is ubiquitous in distribution. Its widespread presence has been reported from almost all the Asian countries (Ahmed et al. 2013; Bekele et al. 2011). This species consists of complex variants as it does not show uniformity in biology and host range and does not work as a single bacterium. Because of its diversified and complex nature, it is therefore characterized and divided into races, biovars, groups, subraces and strains (Hayward 1964; He, L. et al. 1983; Kelman et al. 1994).

The findings of the present study showed an overall incidence of 10% and prevalence of 76% of the disease in the country. Differences in the incidence and prevalence of bacterial wilt were observed all over the country. The following studies show

that its incidence has been reported in many countries. A disease incidence of about 30% has been recorded from Bangladesh on aubergine. On potato and chili in the main chili and potato growing areas in Ethiopia, the incidences of 25% and 55% have been reported respectively (Bekele et al. 2011). The bacterium has a wide host range and causes infections on many crops and vegetables of economic significance. Its incidence has also been reported on different crops. An incidence of about 6% has been recorded on aubergine, 17% on hot peppers, 11% on potatoes, 22% on sweet pepper, and 13% on tomatoes (Begum et al. 2012; Shahbaz et al. 2015). The present study also showed that biovar 3 was dominant in all the zones of the country whereas biovar 4 was reported only from four ecological zones. Similarly, consistent with the current findings, the biovars 3 and 4 were found dominant in Guangdong in previous reports (Zeng and Dong 1995). Differences in the incidence and prevalence of bacterial wilt in different regions and on different crops are ascribed to an assortment of factors viz. the variations in *R. solanacearum* isolates, differences in environmental and edaphic factors in various ecological zones and countries.

In the present study, the highest incidence (19.5%) was recorded from Indus delta whereas it was the lowest (5%) in western dry mountains. Indus delta and southern irrigated plains are famous for chili cultivation in the province of Sindh where chili is mostly and continuously grown. Multiple cropping and interplanting are commonly followed in these zones. Majority of the growers in these areas possess vast landholdings and chili is cultivated as the main crop. Besides chili, solanaceous crops such as tomato, potato, eggplant, etc. are also cultivated on large scale. In these areas, the cultivation of crops is mostly done by the tenants in a traditional way. The certified seed of chili is usually not available to majority of the farmers and the same germplasm consisting of few indigenous cultivars is grown every year. On the contrary, the growers of Sulaiman Peidmont, wet mountains and western dry mountains own small landholdings and practice different cropping pattern. In certain parts of these regions, chili is not cultivated year after year. In these regions the amount of annual precipitation is low excepting wet mountains. There is no practice of intensive cropping in these areas. It is proven that the areas where interplanting and intensive and repeated cropping are practiced, the severity of bacterial wilt increases every year (Persley et al. 1985).

Temperature had a strong effect on the infection of *R. solanacearum*, incidence and development of wilt symptoms in many crops (Bittner et al. 2016; Singh et al. 2014; Tjou-Tam-Sinet al. 2017; van der Wolf et al. 2022). There are reports which corroborated that the severity of the bacterium increased in areas where the temperature ranged from 24 to 35 °C (Lemay et al. 2003) as temperature has been found to be a crucial governing factor in the pathogenesis of the disease, interaction between host and parasite, reproduction and survival of the pathogen in the soil. In Guangdong, China, in the areas with high temperatures i.e., above 30 °C for about 6 months, bacterial wilt appeared seriously on host plants (He, L. et al. 2008; Liu et al. 2009; She et al. 2012, 2013). The differences in soil temperature have more impact on the pathogenesis, development and severity of the disease than that in the air (Gallegly Jr. and Walker 1949). Although the increase in the severity of bacterial wilt has a direct relationship with the soil temperature yet it differs from cultivar to cultivar (Aslam et al. 2017a, b; Grieve 1943; Kelman 1953; Mew and Ho 1977; Tajul et al. 2011; Vaughan 1944). The degree of movement of the bacterium in the stems relies mainly on moisture and the temperature in the soil optimally at 32 °C (Kelman 1953). It was also reported that the bacterium did not produce symptoms of bacterial wilt when the temperature was less than 21 °C (Vaughan 1944). In Pakistan, such specified favorable conditions of moisture and temperature are predominant during the period of monsoon and greatly favor the reproduction of the pathogen and development of disease and validate the findings of Linus et al. (2004). The bacterium can thrive well in every kind of soil whether clayey or sandy and alkaline or saline however, the saline soils with pH < 7.0 favor the pathogen greatly.

One of the major causes of the dominance of the bacterium in chili growing areas of the country particularly in the province of Sindh is multiple cropping. The solanaceous vegetables, viz. tomato, potato, eggplant, etc., are cultivated all year round in the same land, which are good hosts of the bacterium allowing the bacterium to survive well throughout the year. Banana, extensively cultivated in the province of Sindh, is another good host of the bacterium and facilitates the continuous survival and dissemination of *R. solanacearum*. Another factor which contributes to its long survival, development and wide spread is the lack of awareness of the growers about the bacterium. They are unaware of the processes involved in the infection, reproduction and distribution of the bacterium.

The pathogen can survive both in water as well as in the soil. Rainy seasons and high moistures in the soil are responsible for high disease intensity. *Ralstonia solanacearum* multiplies and thrives effectively when moisture contents in the soil ranges from 0.5 to 1 bar whereas the moisture range of –5 to –15 bar in the soil becomes unpropitious for the survival and

reproduction of the bacterium. The environmental and edaphic factors prevalent in the Indus delta, southern irrigated plains and sandy deserts of the Sindh and Punjab provinces are favorable for the bacterium causing disease incidence and severity high and confirm the results of Nesmith and Jenkins (1985). The bacterium is mainly dispersed through infected propagating material (infected potato tubers and seedlings raised in infested soils) (Olsson 1976), noncertificated seed and infected farm machinery. The inoculum of the bacterium increases in the soil because of recurrent and continued cultivation of same crops and interplanting with susceptible hosts which result in severity of the disease.

CONCLUSION

The study concludes that bacterial wilt incited by the bacterium *R. solanacearum* is widespread all over the country with variable magnitudes and demands strict vigilance and management strategies. Biovar 3 was found dominant in all the zones of the country whereas biovar 4 was reported only from four ecological zones. This is first study of its kind which documents the incidence, prevalence and biovar determination of *R. solanacearum* infecting chili from different areas of Pakistan.

AUTHORS' CONTRIBUTION

Conceptualization: Aslam M. N. and Mukhtar T.; **Methodology:** Aslam M. N. and Mukhtar T.; **Investigation:** Aslam M. N. and Mukhtar T.; **Writing – Original Draft:** Aslam M. N. and Mukhtar T.; **Writing – Review and Editing:** Mukhtar T.; **Supervision:** Mukhtar T.

DATA AVAILABILITY STATEMENT

Data will be made available upon request.

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