Distributional variability of bacterial wilt of chili incited by *Ralstonia solanacearum* in eight agro-ecological zones of Pakistan

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Background. Bacterial wilt caused by *Ralstonia solanacearum* is one of the major constraints in the production of chilies in Pakistan. As the information regarding distribution and prevalence of *R. solanacearum* is exiguous, the present studies were conducted during 2014-15 to determine the incidence and prevalence of *R. solanacearum* in the major chili growing areas from different agro-ecological zones of Pakistan. **Results.** The overall incidence and prevalence of *R. solanacearum* in the country was found to be 10% and 76% respectively. Of the four provinces, maximum disease incidence of 16.4% was recorded in Sindh province followed by Punjab and Khyber Pakhtoonkhwa showing 11.4% and 7% disease incidences respectively and the minimum incidence of 4.9% was observed in the province of Baluchistan. As regards prevalence, the same pattern was observed. Out of 8 agro-ecological 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% was found in Western dry mountains. The disease incidence in other zones ranged between 5.4 and 14.1%. Similar trend was noticed regarding prevalence being the maximum in Indus delta (100%) followed by Southern irrigated plains (90%). Out of 114 *R. solanacearum* strains, 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 Punjab and Sindh provinces only. Similarly, biovar 3 was observed from all the eight agro ecological zones and found to be predominant. On the other hand, biovar 4 was recorded from four agro ecological zones. **Conclusions.** The study provides first comprehensive report about the distribution of bacterial wilt of chilies in all the agro ecological zones of the country. The disease has been found fairly distributed in the country with varying intensities warranting stringent surveillance and control measures to minimize yield losses.

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Abstract

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Results. The overall incidence and prevalence of *R. solanacearum* in the country was found to be 10% and 76% respectively. Of the four provinces, maximum disease incidence of 16.4% was recorded in Sindh province followed by Punjab and Khyber Pakhtoonkhwa showing 11.4% and 7% disease incidences respectively and the minimum incidence of 4.9% was observed in the province of Baluchistan. As regards prevalence, the same pattern was observed. Out of 8 agro-ecological 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% was found in Western dry mountains. The disease incidence in other zones ranged between 5.4 and 14.1%. Similar trend was noticed regarding prevalence being the maximum in Indus delta (100%) followed by Southern irrigated plains (90%). Out of 114 *R. solanacearum* strains, 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 Punjab and Sindh provinces only. Similarly, biovar 3 was observed from all the eight agro ecological zones and found to be predominant. On the other hand, biovar 4 was recorded from four agro ecological zones.

Conclusions. The study provides first comprehensive report about the distribution of bacterial wilt of chilies in all the agro ecological zones of the country. The disease has been found fairly distributed in the country with varying intensities warranting stringent surveillance and control measures to minimize yield losses.

Key words: Surveillance; Bacterial wilt; biovar; distributive variations; agro-ecological zones.

Introduction

Bacterial wilt caused by *Ralstonia solanacearum* is a serious threat to solanaceous crops both in temperate and tropical regions of the world (Hayward, 1991). Bacterial wilt is ubiquitous in distribution with varying proportions. In Bangladesh up to 31% disease incidence has been reported on egg plant (Hussain et al., 2005). 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 have to be demolished due to quick spread of the pathogen all over the Peruvian Jungle (French & Sequeira, 1968).

The pathogen has been reported to invade over 450 plant species from 54 botanical families (Wicker et al., 2007) and incur huge yield losses. 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 (Darong et al., 1981; Sitaramaiah & Sinha, 1983). In India, the bacterium caused complete failure of tomato crop. Widespread losses on potato have also been reported in Greece (Zachos, 1957). 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 (Chen, 1984; Sitaramaiah and Sinha, 1984).

Bacterial wilt has also been reported from Pakistan infecting a large number of host plants. It was first reported from Pakistan in 1989 (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) as compared to Morroco (22.04 tons/h) 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. (Hayward, 1964; He et al., 1983; Kelman et al., 1994).

Environmental factors have traditionally been considered to have the major impact on disease development, spread and distribution. Even if a susceptible host and a virulent pathogen are present in a certain locality, serious disease will not occur unless the environment favors its development. This includes both the aerial and soil (edaphic) environment. Environmental factors may exclude a pathogen from, or greatly reduce its fitness in a particular part of the potential range of a crop. Weather conditions have a great impact on disease development and have been intensively studied as predictors of disease outbreaks. Moisture is the most important environmental factor influencing disease outbreaks caused by fungi and bacteria. Temperature has a much greater effect on disease development in temperate and tropical climates. Soil fertility can also affect development of both soil- and air-borne disease.

In Pakistan, little work has been done about this pathogen (Burney, 1995; Burney & Ahmed, 1997; Burney et al., 1999) and the information regarding its incidence and prevalence particularly on chili crop in different agro-ecological 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 agro ecological zones. The study will help the farmers in designing control strategies against the bacterium accordingly.

Materials & methods

Description of the studied areas

The studies on the determination of incidence and prevalence of *Ralstonia solanacearum* inciting bacterial wilt of chilies and distribution of biovars of *R. solanacearum* were conducted in eight agro ecological 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 Pothowar region followed by Indus plain, 322 km wide and 1287 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 agro-ecological zones. The salient features of these agro-ecological zones of the country have been described in Table 1 and 2.

Distribution of *Ralstonia Solanacearum*

For recording incidence of bacterial wilt in Pakistan, an extensive survey of chili was conducted during 2014-15 in 14 major chili cultivating districts falling in 8 agro-ecological zones of Pakistan (Table 3 and Figure 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 immunostrip in the field. The incidence of bacterial wilt of each site was calculated as described by Fateh et al. (2017). Similarly, the incidences from all the districts,

agro-ecological zones, provinces and finally the whole country were calculated. Disease prevalence of bacterial wilt in each district, agro-ecological zone, province and the country was also reckoned as described by Fateh et al. (2017).

Symptomatological confirmation of bacterial wilt

The wilted plants were identified by the characteristic symptoms of the disease. These symptoms include 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 include progressive discoloration of vascular tissues mainly xylem and appearance of slimy viscous ooze when the stems were cut transversely.

Serological Confirmation of *Ralstonia solanacearum* in wilted plants

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

Collection of *R. solanacearum* strains

A total of 114 strains of *R. solanacearum* associated with chili were collected from 14 major chili growing districts falling under eight agro-ecological 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 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 agro-ecological zones as described below

Isolation from soil

The bacterium was 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 10^7were made by adding requisite amount of distilled water. By using micro pipette, 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 28ºC for 48 h for bacterial growth (Englebrecht, 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 minutes with continuous shaking in a shaker at room temperature. The bacterial suspension (100 µl) from each sample was streaked separately on the TTC (Triphenyle Tetrazolium Chloride) medium, spread uniformly and incubated as mentioned above (Hugh & Leifson, 1953).

Purification of *R. solanacearum*

For obtaining 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 TZC, Cyclohexamide, Bacitracin, and Penicillin to avoid contamination.

Confirmation of *R. solanacearum* strains

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

Hyper Sensitive reaction (HR)

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 leaves mesophyll of tobacco plants by using sterilized syringe. The distilled water was used as a positive control. Each strain 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 (HR) in the inoculated areas of the leaves. The confirmed purified strains were coded accordingly.

Characterization of *R. solanacearum*

The isolates were characterized morphologically by their growth patterns (mucoid and non mucoid growth) and biochemically by employing various biochemical tests viz. gram reaction, Catalase activity, Levan Production (Schaad, 1980), KOH loop test (Suslow et al., 1982), Oxidase Activity (Kovacs, 1956), Lipase Activity, Pigment Production (King et al., 1954), Arginine dihydrolase reaction (Thronly, 1960), Gas production (Van den Mooter, 1987), Oxidation and Fermentation Activity (Hayward, 1964).

Molecular confirmation

The DNA from the 114 purified strains was extracted, quantified and amplified by using the primer pair JHFegl: 5'GACGATGCATGCCGCTGGTCGC 3' and JHRegl: 5' CACGAACACCACGTTGCTCGCATTGG 3'. Each PCR amplification reaction 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 µM) of MgCl2, 1.0 µl (10 µM) 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 UV light after ethidium bromide staining. All strains yielded a 750-bp band that corresponds to *Ralstonia solanacearum*

Identification of Biovars

The bacterial strains were identified into biovars on the basis of utilization of different sugars. One gram of each disaccharides (maltose, cellobiose, lactose) and hexsoe 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 @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 et al., 1983)

Results

The overall incidence of *Ralstonia solanacearum* in the country was found to be 10% and the prevalence of 76% was recorded. Of the four provinces, maximum disease incidence of 16.4% was recorded in Sindh province followed by Punjab and Khyber Pakhtoonkhwa showing 11.4% and 7% 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%) in the province of Sindh followed by Punjab and Khyber Pakhtoonkhaw provinces giving 84% and 65% disease prevalence. On the contrary, the minimum disease prevalence of 60% was observed in Baluchistan province as shown in Figure 2 and 3.

198 Out of 8 agro-ecological zones, the maximum disease incidence of 19.5% was observed in
199 Indus delta followed by Sandy deserts (14.1%) while the minimum disease incidence of 5% was
200 found in Western dry mountains. The disease incidence in other zones ranged between 5.4 and
201 14.1% as shown in figure 4. In case of disease prevalence, the maximum disease prevalence was
202 observed in Indus delta which was found 100% followed by 90% of Southern irrigated plains
203 while the minimum disease prevalence of 70% was recorded in Western dry mountains and
204 Suleiman piedmont. The prevalence ranged between 70 and 86.6% in other agro ecological zones
205 as shown in Figure 5.
206 Of the 14 districts, the maximum disease incidence of 22% was observed in district Badin
207 followed by district Thatta (17%) while the minimum disease incidence was observed in district
208 Loralai of 4.40 %. The disease incidence was found variable in the remaining districts as shown
209 in figure 6. Similarly, the maximum disease prevalence was found in Thatta, Badin and
210 Mirpurkhas districts which were 100% while the minimum disease prevalence of 60% was
211 observed in Karak, Loralai and Barkhan districts. The rest of the districts had disease prevalence
212 ranging from 70 to 90% (Figure 7).

213 **Identification of Biovars**

214 Out of 114 *R. solanacearum strains*, 92 (81%) were identified as Biovar 3, while the
215 remaining 22 (19%) were recognized as Biovar 4. Biovar 3 was recorded from all the four
216 provinces and was found to be predominant in all the provinces while Biovar 4 was found in the
217 Punjab and Sindh provinces only as shown in Table 4.

218 Similarly, biovar 3 was observed from all the eight agro ecological zones and found to be
219 predominant. On the other hand, biovar 4 was recorded from four agro ecological zones. The
220 zone wise and district wise distribution of biovars 3 and 4 is given in Table 5.

Discussion

R. solanacearum is widespread in warm temperate, tropical and subtropical regions of the world. In Asia it has been reported from almost all the countries (Bekele et al., 2011; Ahmed et al., 2013). *R. solanacearum* does not have uniform biology, host range and act as complex variants, as it does not behave as single bacterium that is why it is described into biovars, races, groups, sub-races and strains.

In the present studies an incidence of 10% and prevalence of 76% of bacterial wilt of chilies caused by *R. solanacearum* was recorded in the country. Incidence and prevalence varied among provinces, agro-ecological zones and fourteen major chili growing districts of the country. Variations in the incidence of bacterial wilt are attributable to the diversity of *R. solanacearum* strains, variations in soil types in different agro ecological zones.

The maximum disease incidence of 19.5% was recorded from Indus delta while the minimum of 5% was recorded from western dry mountains. The Indus delta and southern irrigated plains are the major chili growing areas from Sindh province with repeated cultivation of chili crop. Multi cropping and intercropping are common practices in these areas. The farmers in these zones have large land holdings and chili is the major crop. In addition to chili other solanaceous vegetables are also widely grown. The crop is mainly grown by the tenants who do not use certified seed. The same germplasm comprising few local varieties is cultivated years after year. On the other hand farmers of Western Dry Mountains, Wet Mountains and Sulaiman Peidmont have small land holdings and do not follow the same cropping pattern. Chili is also not grown repeatedly in certain areas in these zones. These zones except wet mountains receive low rainfall. There is no intensive cropping in these zones. There are reports that the areas with inter-cropping and repeated cropping practices result in increase in bacterial wilt severity each year (Persley et al., 1985). It is well documented that *R. solanacearum* is more severe in areas having temperature range of 24-35°C (Johnson, 2003; Lemay et al., 2003) as temperature plays an

246 important role in host-pathogen interaction as well as the survival of pathogen in the soil. The
247 variations in soil temperature are more influential in disease initiation and severity as compared
248 to variation in air temperature (Gallegly & Walker, 1949). The increase in bacterial wilt disease is
249 directly proportional to soil temperature but it varies from cultivar to cultivar (Grieve, 1943;
250 Vaughan, 1944; Kelman, 1953; Mew & Ho, 1977; Tajul et al, 2011). The movement rate of the
251 pathogen in the stem is directly dependent on soil temperature (32ºC opt.) and moisture (Kelman,
252 1953). Vaughan, 1944 reported that at temperature below 21ºC, bacterial wilt symptoms did not
253 develop.

254 In Pakistan such conditions prevail during the monsoon season which favor the disease
255 development. These conditions favor the multiplication of the bacterium which supports the
256 claim of Linus et al. (2004) where they reported the positive relationship between bacterial wilt
257 incidence and moisture. The pathogen can survive in all types of soils including sandy and clay
258 types. This bacterium can also survive in soils both acidic and basic in nature but it prefers the
259 acidic soils with pH <7.0.
260 The main reasons for its dominance are the cultivation of multiple crops. The solanaceous
261 vegetables are grown throughout the year almost all are the hosts of *R. solanacearum*. Banana
262 which is a good host of *R. solanacearum* is widely grown in Sindh province and helps in the
263 spread of the bacterium. The other reasons for its spread and development include incognizance
264 of the farmers about this pathogen. They are incognizant of the mechanism of invasion and
265 spread of the bacterium.
266 The bacterium has the ability to survive in the soil and irrigation water. High soil
267 moisture and wet or rainy seasons are contributory factors to high disease severity. The bacterium
268 reproduces and grows well at soil moisture of 0.5 to 1 bar which is favorable for the reproduction
269 and survival of *R. solanacearum* while soil moisture of -5 to -15 bar becomes non-conducive for
270 the pathogen (Nesmeth & Jenkins, 1985). The soil and environmental conditions prevailing in

Indus Delta, Southern Irrigated plains and sandy deserts of Sindh and Punjab provinces favor *R. solanacearum* and are attributable to its high incidence and severity of the this disease and support the findings of Nesmetho and Jeng (1985).

The other major sources of dispersal of this bacterium are infected planting material (infected potato tubers and seedlings raised in infested soils) (Olsson, 1976a, b) use of uncertified seed and contaminated farm implements. The inoculum of the bacterium builds up in the soil due to repeated and continuous growing of same crops and intercropping with susceptible hosts which results in severity of the disease.

Conclusions

The study provides first comprehensive report about the distribution of bacterial wilt of chilies in all the agro ecological zones of the country. The disease has been found fairly distributed in the country with varying intensities warranting stringent surveillance and control measures to minimize yield losses. 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 Punjab and Sindh provinces only. Similarly, biovar 3 was observed from all the eight agro ecological zones and found to be predominant. The information will be helpful in designing control strategies and in breeding programs to develop new resistant varieties accordingly.

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Table 1(on next page)

Meteorological parameters of eight agro-ecological zones of Pakistan

Table 1: Meteorological parameters of eight agro-ecological zones of Pakistan

S. No.	Agro-Ecological Zone	Districts	Temperature (°C)		RF (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

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Table 2(on next page)

Soil conditions and crops of eight agro-ecological zones of Pakistan

Table 2: Soil conditions and crops of eight agro-ecological zones of Pakistan

S.No	Agro-Ecological Zone	Soil Type	Soil pH	Organic matter (%)	Major Crops
1	Indus Delta	Clayey and silty	7.6-8.2	0.5-0.8	Cotton, Sugar cane, Wheat, Maize, Millet, Barley and Vegetables
2	Southern Irrigated Plain	Silty and sandy loam	6.8-7.2	0.4-0.5	Cotton, Sugar cane, Wheat Maize, Millet, Barley and Vegetables
3	Sandy desert	Sandy soils and moving dunes with strips of clayey soils	7.8-8.3	0.4	Cotton, Sugar cane, Wheat Maiz Millet, Barley, xerophytic vegetation and Vegetables
4	Northern Irrigated Plain	Sandy loam, clay loam, silt loam and 15 % saline -sodic	7.5-7.8.2	04-0.6	Cotton, Sugar cane, Wheat, oilseeds, rice
5	Barani areas	Non-calcareous to moderately calcareous, silt loams and with west southern part mainly calcareous	7.5-7.7	<0.5	Sorghum, Millet, Maize, Pulses, Ground nut, wheat and vegetables
6	Wet Mountains	Silt loam to silty clays, non-calcareous to slightly calcareous	7.5-8.1	0.4-0.6	Maiz, Wheat, Fruits (Apples). Olives Forests and vegetables
7	Western Dry Mountains	Loamy, deep and calcareous	8.3	0.3-0.5	Wheat, Grazing, apples, plums, apricots, grapes, peaches and vegetables
8	Sulaiman Piedmont	Silt loam, deep and strongly calcareous	8.5	0.3-0.4	Xerophytic vegetation, grasses, wild olives, fruits, vegetable and wheat

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Table 3(on next page)

Area under chili cultivation in the surveyed districts falling in eight agro-ecological zones of Pakistan

Table 3: Area under chili cultivation in the surveyed districts falling in eight agro-ecological zones of Pakistan

Districts	Area (in acres)	Agro-ecological zone
Thatta	4342	
Badin	15216	**Indus delta**
Total area	**19558**	
Umerkot	35445	
Mirpurkhas	12821	**Southern Irrigated Plains**
Total area	**48266**	
Sanghar	2560	
Bahawalpur	760	**Sandy Deserts**
Total area	**3320**	
Multan	1800	
Pakpattan	1120	**Northern Irrigated Plains**
Kasur	1200	
Total area	**4120**	
Attock	615	**Barani Areas**
Total area	**615**	
Nowshehra	1350	**Wet Mountains**
Total area	**1350**	
Karak	1200	
Loralai	933	**Western Dry Mountains**
Total area	**2133**	
Barkhan	4077	**Suleiman Piedmont**
Total area	**4077**	

Table 4(on next page)

Distribution of biovars in the four Provinces of Pakistan

Table 4: Distribution of biovars in the four Provinces of Pakistan

Province	Total Isolates	%age	Biovar III	%age	Biovar IV	%age
Sindh	47	41	33	70	14	30
Punjab	42	37	34	81	08	19
Khyber	13	11	13	100	-	-
Baluchistan	12	11	12	100	-	-
Pakistan	**114**	**-**	**92**	**81%**	**22**	**19%**

Table 5(on next page)

Distribution of biovars in different agro ecological zones and districts of Pakistan

1 **Table 5: Distribution of biovars in different agro ecological zones and districts of Pakistan**

Agro-ecological zone	District	Total isolates	%age	Biovar3	%age	Biovar4	%age
Indus delta	Thatta	10	8.77%	06	60%	04	40%
	Badin	10	8.77%	07	70%	03	30%
	Total	**20**	**17.54%**	**13**	**65%**	**07**	**35%**
Southern irrigated plains	Mir Pur Khas	10	8.77%	08	80%	02	20%
	Umer Kot	08	7%	05	62.5%	03	37.5%
	Total	**18**	**15.78%**	**13**	**72.2%**	**05**	**27.8%**
Sandy deserts	Bahawalpur	08	7%	07	87.5%	01	12.5%
	Sanghar	09	7.89%	07	77.8%	02	22.2%
	Total	**17**	**14.9%**	**14**	**82%**	**03**	**18%**
Northern irrigated plains	Multan	08	7%	06	75%	02	25%
	Pakpattan	09	7.89%	06	66.7%	03	33.3%
	Kasur	09	7.89%	07	77.8%	02	22.2%
	Total	**26**	**22.8%**	**19**	**73%**	**07**	**27%**
Barani areas	Attock	08	7%	08	100%	-	-
	Total	**08**	**7%**	**08**	**100%**	**-**	**-**
Wet mountains	Nowshera	07	6.14	07	100%	-	-
	Total	**07**	**6.14**	**07**	**100%**	**-**	**-**
Western dry mountains	Karak	06	5.26%	06	100%	-	-
	Loralai	06	5.26%	06	100%	-	-
	Total	12	10.52%	12	100%	-	-
Suleiman piedmont	Barkhan	06	5.26%	06	100%	-	-
	Total	**06**	**5.26%**	**06**	**100%**	**-**	**-**
Pakistan	**Grand total**	**114**	**-**	**92**	**81%**	**22**	**19%**

2

3

4

Figure 1(on next page)

Map showing agro-ecological zones of Pakistan

Agro Ecological Zones of Pakistan

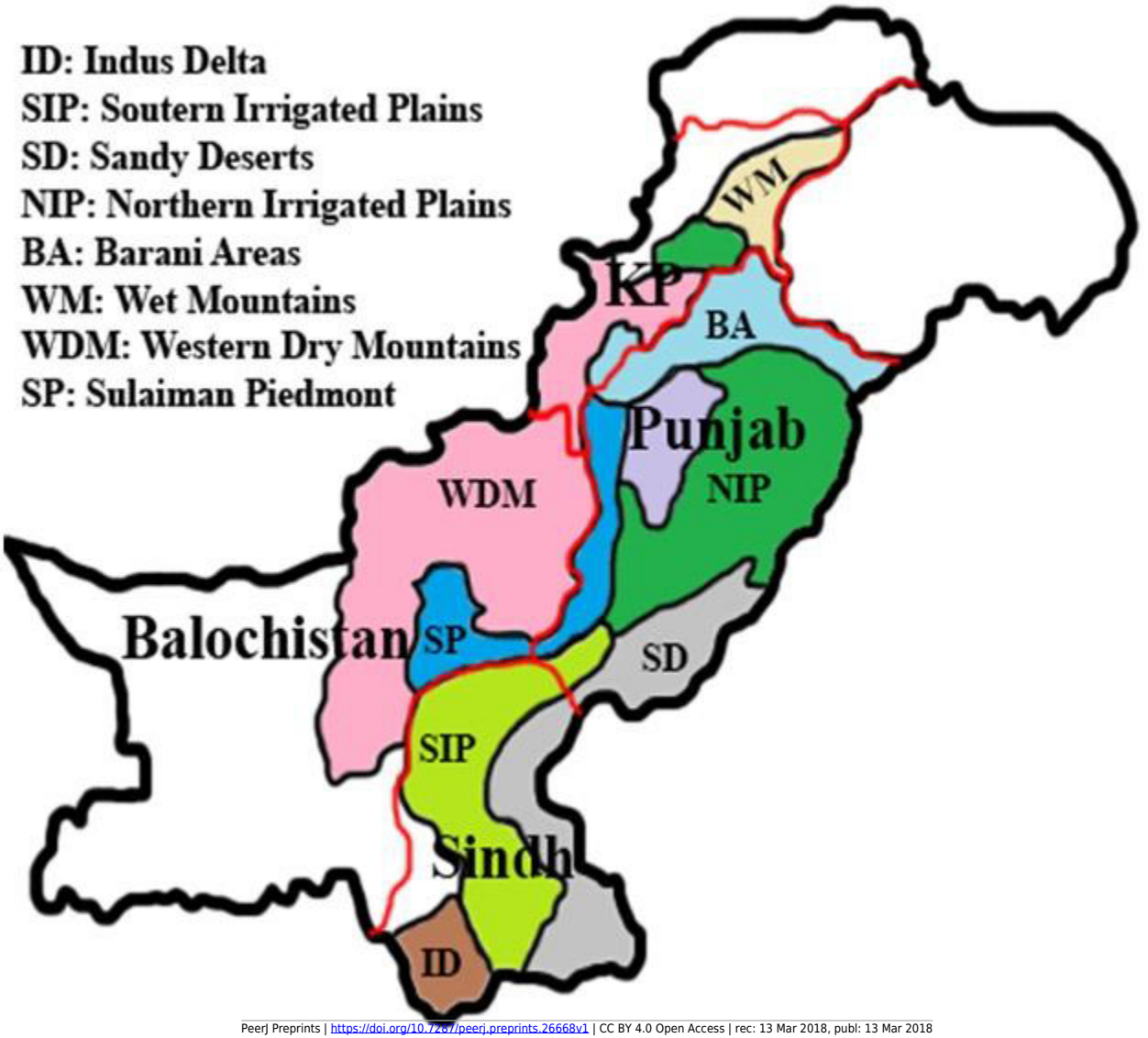

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Figure 2(on next page)

Incidence of bacterial wilt in four provinces of Pakistan

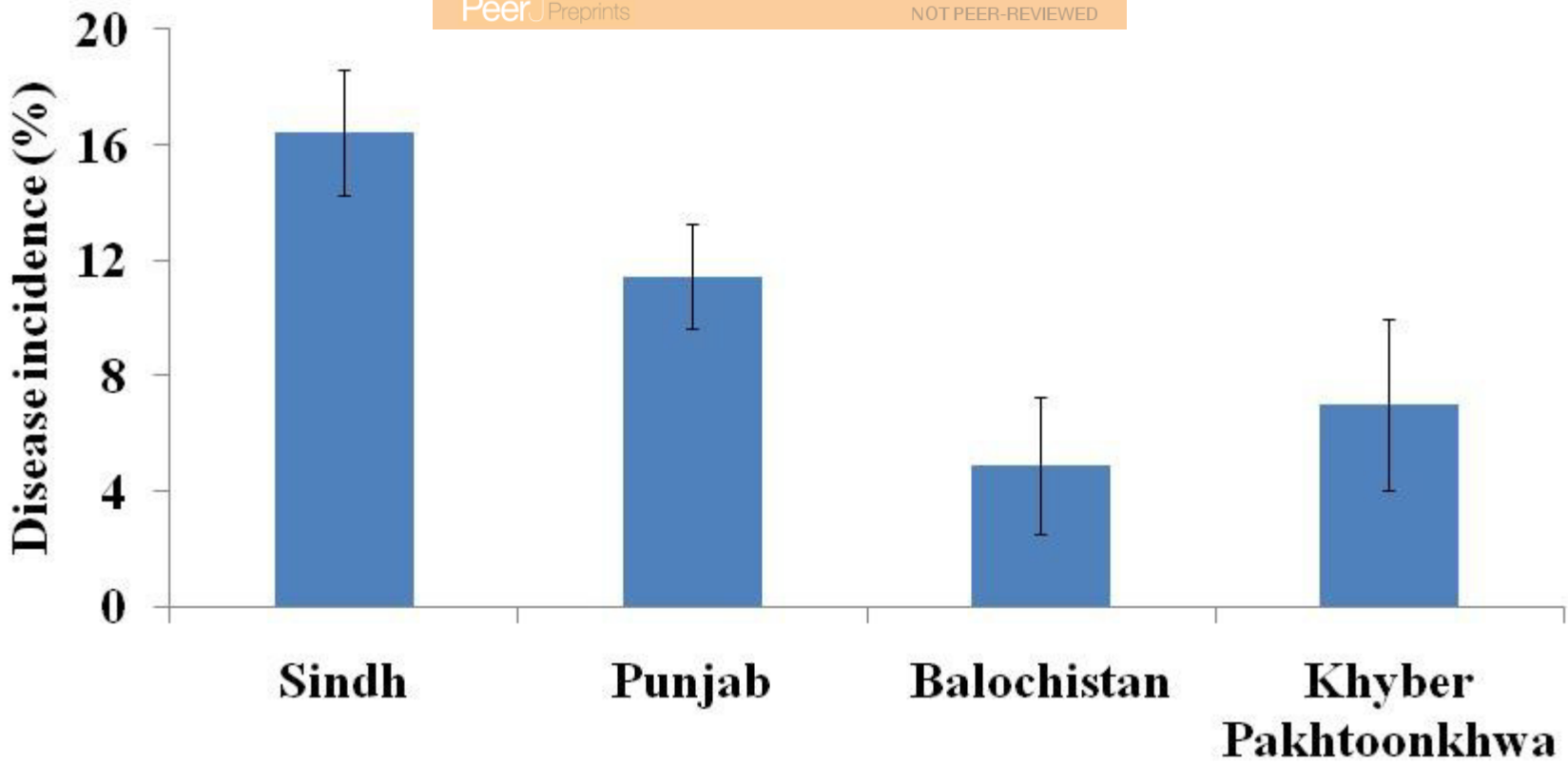

Fig. 2

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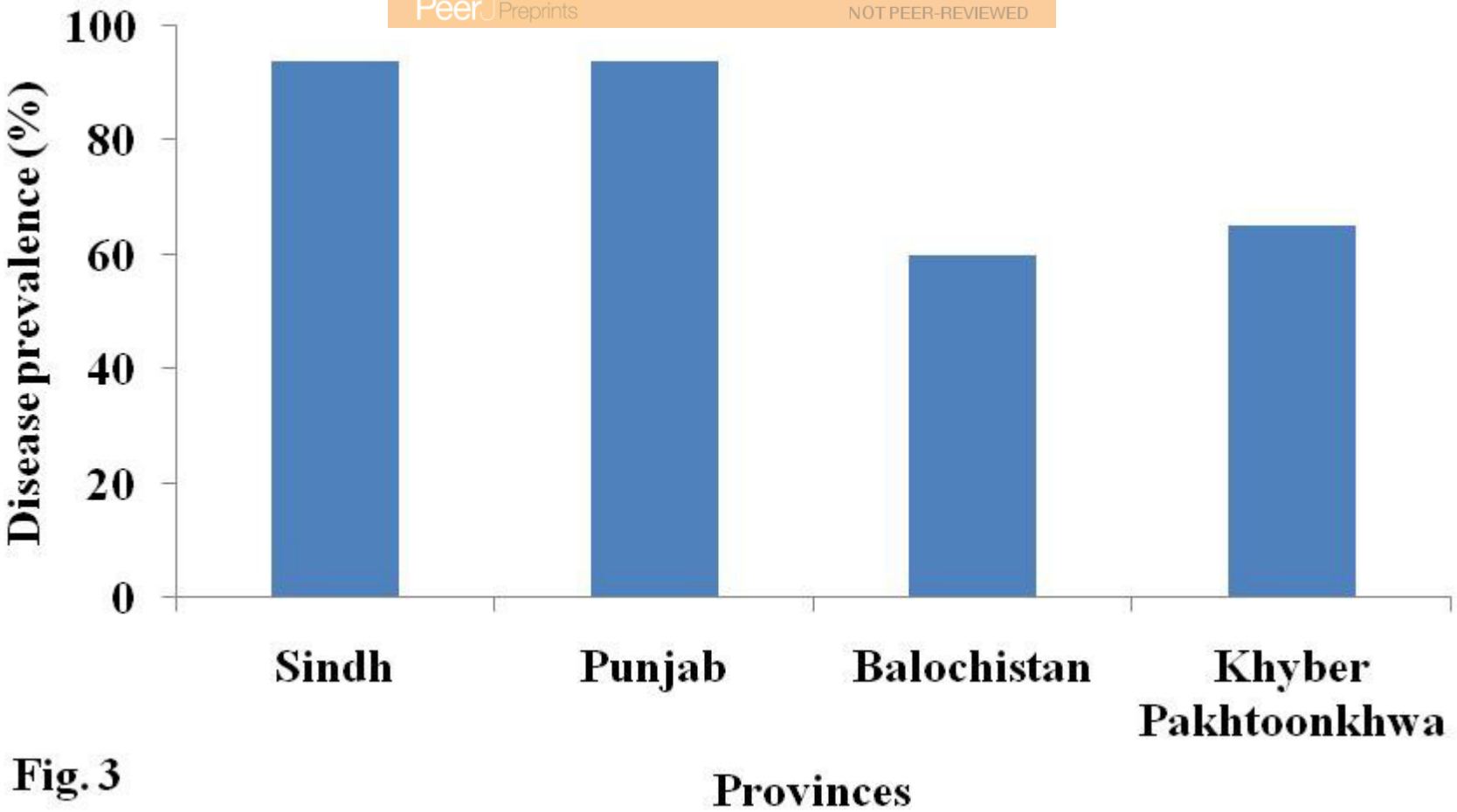

Fig. 3

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Figure 4(on next page)

Incidence of bacterial wilt disease in eight agro-ecological zones of Pakistan

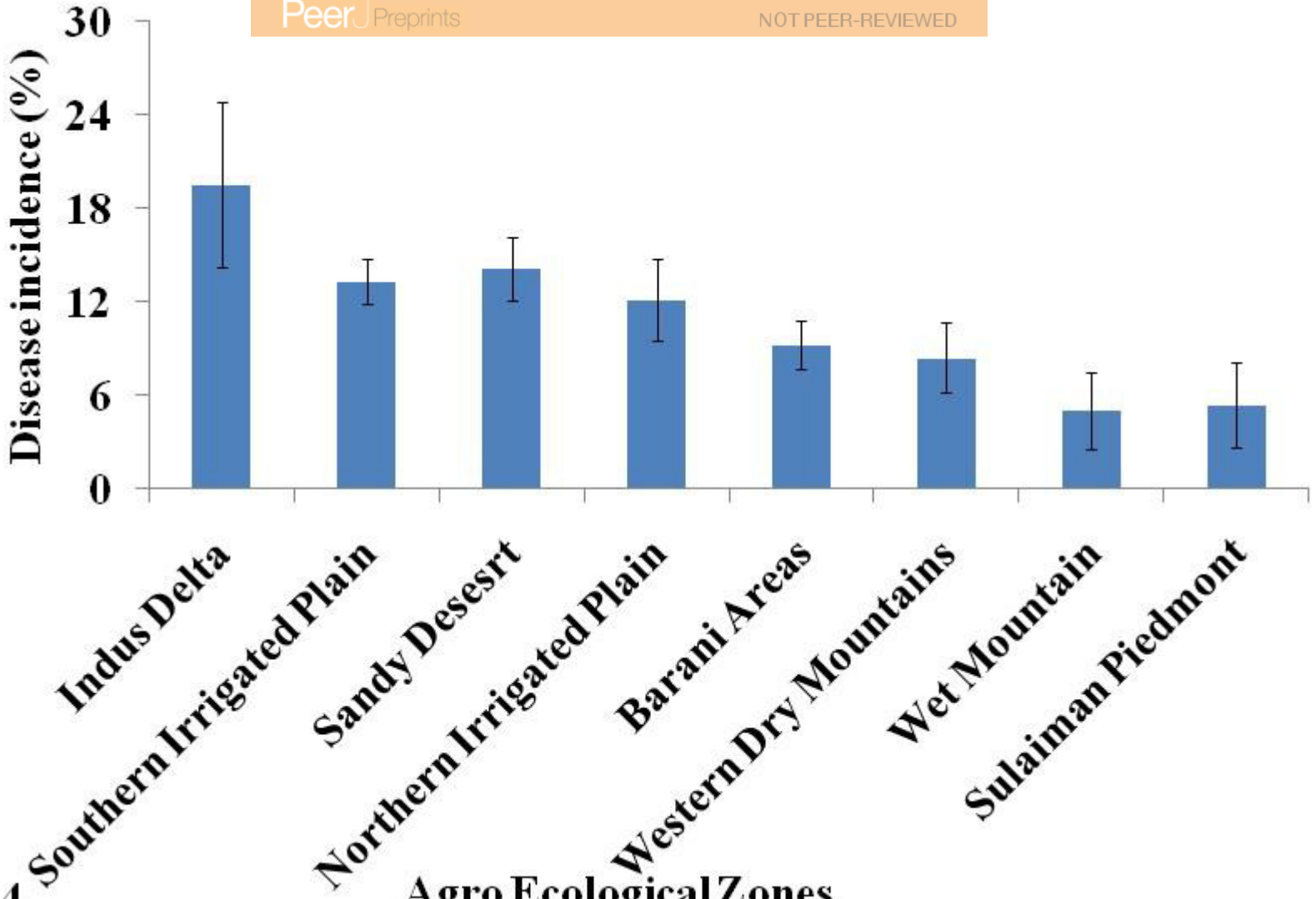

Fig. 4

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Figure 5(on next page)

Prevalence of bacterial wilt disease in eight agro-ecological zones of Pakistan

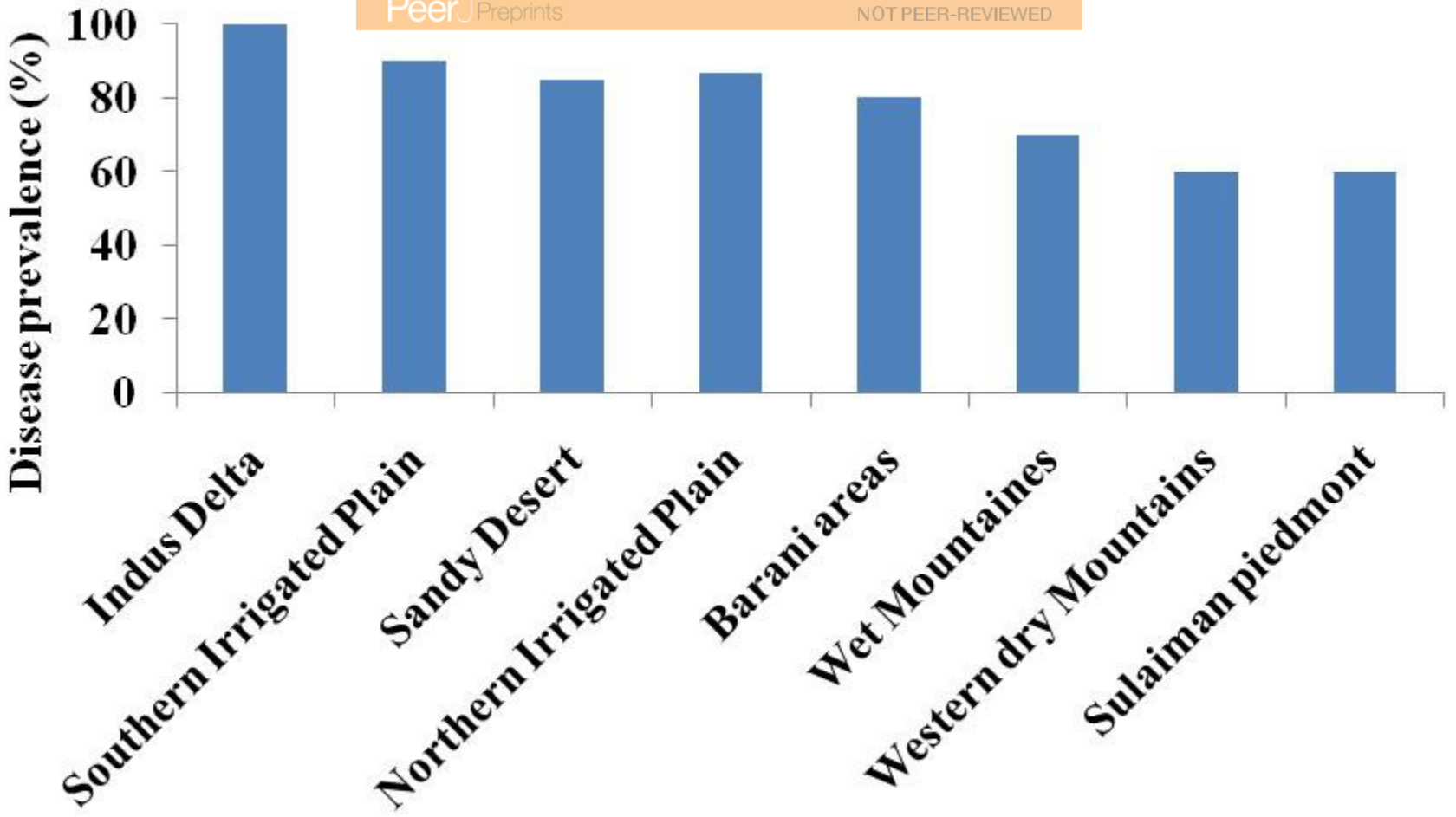

Fig. 5

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Figure 6(on next page)

Incidence of bacterial wilt disease in fourteen districts of Pakistan

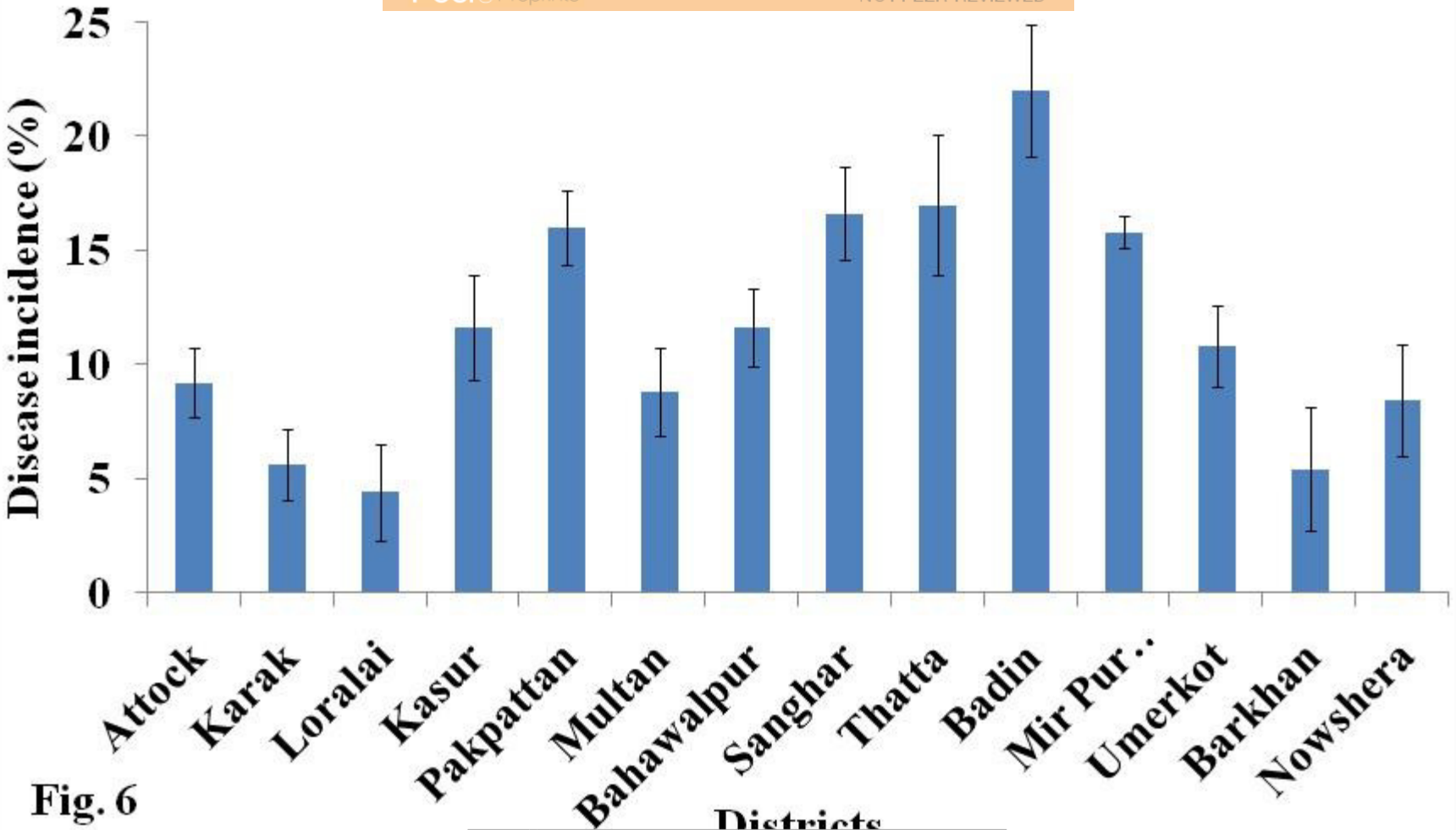

Fig. 6

Figure 7(on next page)

Prevalence of bacterial wilt disease in fourteen districts of Pakistan

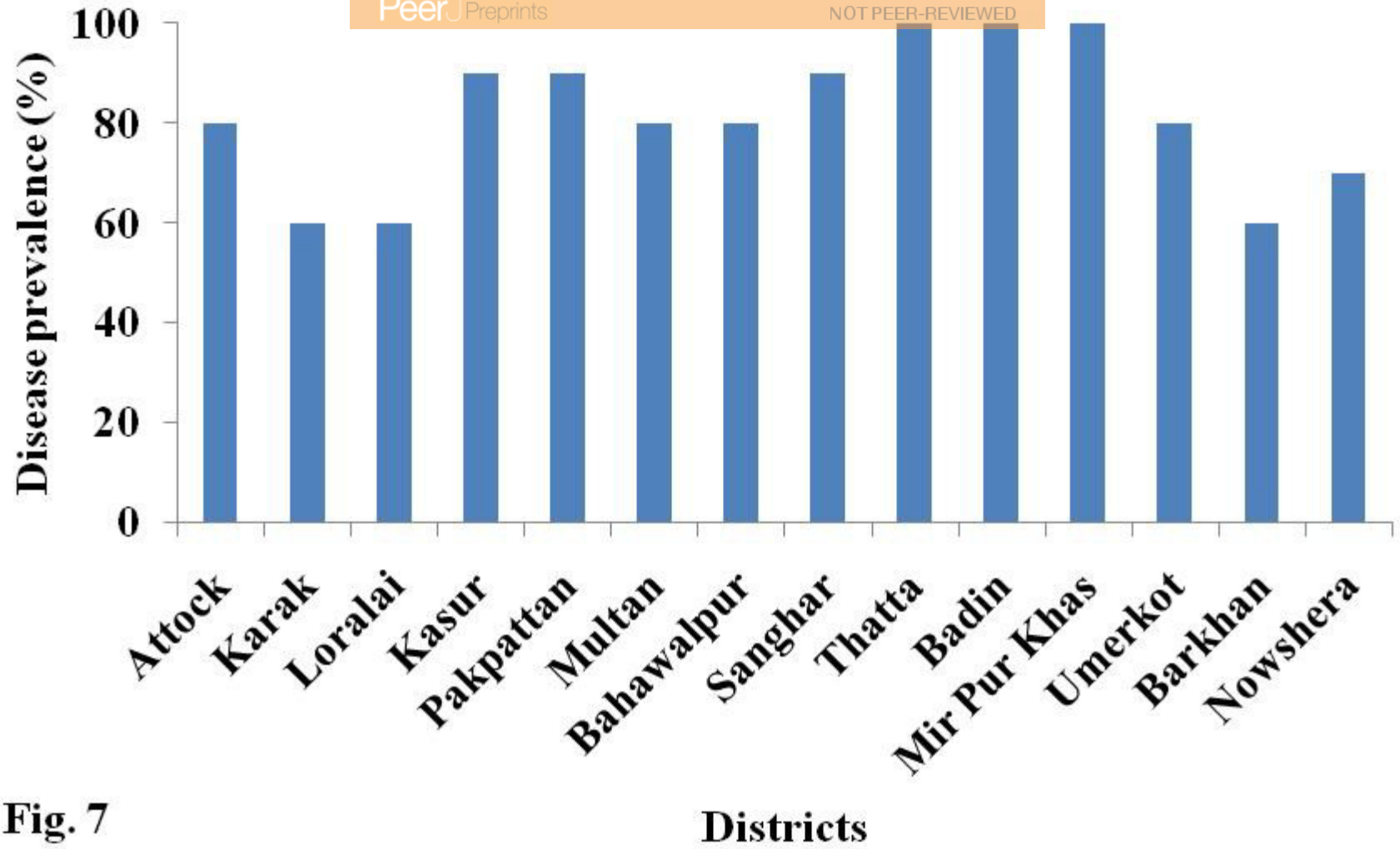

Fig. 7

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