

THE SCENARIO OF LENTIL ANTHRACNOSE IN THE PUNJAB, PAKISTAN

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Abstract

Lentil anthracnose disease incited by *Colletotrichum lentis* Damm is one of the most significant fungal threats to lentil (*Lens culinaris* Medikus) cultivation worldwide. No detailed data on lentil anthracnose presence and its impact with reference to Pakistan is available. Thus an extensive two years (2014, 2015) disease survey was carried to figure out the scenario of lentil anthracnose in thirteen major lentil growing districts of the Punjab province namely Rawalpindi, Chakwal, Attock, Jhelum, Gujrat, Sialkot, Narowal, Mianwali, Khushab, Jhang, Bhakkar, Layyah and Muzaffargarh. Consequently, 162 samples (80 during 2014; 82 during 2015 crop season) were collected from private lentil farms and the government research institutes. Two-three random samples depending on farm size were taken. Districts mean disease incidence (DI) and disease severity (DS) was calculated. Disease was found in 100% areas/locations visited. At individual locations, the maximum mean DI 85 % and DS 9 was found at Noorpur Thal (2014), whereas the minimum DI 5 and DS 3 was observed at Bhadder (2014). Year-wise, the maximum districts mean DI of 69 % and DS 7 was noted in Khushab (2014), while the minimum districts mean DI 19% and DS 3 was observed in Gujrat (2014). Based on two years data, the highest districts mean DI 68% & DS 7 was recorded in Khushab followed by Sialkot DI 63% and DS 7, however the lowest DI 21% and DS 3 was observed in Jhang. Locations/districts with the highest disease intensity require appropriate support for lentil anthracnose management.

Key words: Lentil anthracnose, *Colletotrichum lentis*, Incidence and severity, Pakistan.

Introduction

Lentil (*Lens culinaris* Medikus) is an essential part of the diet as a source of protein in many parts of the world, especially South Asian regions, which have a large vegetarian population. Its production has been increasing in Canada, United States of America, Australia and China but has been somewhat constant in India, Pakistan, Turkey, Syria, Iran, Nepal and Bangladesh (Anon., 2014). South Asia has approximately half of the total world area under cultivation of this crop. In Pakistan, lentil is an important pulse (a term commonly used for the dried seed of lentils, chickpeas, peas and edible beans) of frequent daily use, especially in the wake of a growing population; its demand is increasing day by day. It is the second largest grown legume crop after chickpea (Bashir & Malik, 1988), cultivated as a winter season or Rabi crop on an area of 18,220 hectare annually (Anon., 2015). Out of this total area, 77.41% is planted in the province of Punjab in the districts of Rawalpindi, Chakwal, Attock, Jhelum, Gujrat, Sialkot, Narowal and Thal (Bhakkar, Layyah, Muzaffargarh, Khushab, Mianwali and Jhang districts are collectively known as Thal) where 2/3 of the area is sown under rain-fed conditions. About 15,000 tons of lentils with an average yield of 510Kg/ha were harvested during the year 2015. Masoor-93, Markaz-2009, Punjab masoor-2009, NIAB masoor-2006, NIAB masoor-2002, NIA masoor-2005 and Shiraz-96 are the well-known lentil cultivars grown here (A. Bakhsh, Director General (Planning and Development), Pakistan Agricultural Research Council, Islamabad - Personal communication).

Fungal diseases are a major threat to lentil production reducing its yield in many parts of the world (Hamdi & Hassanein, 1996). Crop is vulnerable to a number of diseases, which adversely affects seed yield and quality.

One serious foliar disease is lentil anthracnose incited by fungus *Colletotrichum lentis* Damm (previously *C. truncatum* (Schwein.) Andrus & W.D. Moore) was first reported by Tode (1790). *Colletotrichum* spp., are distributed primarily in tropical and subtropical regions causing anthracnose of a wide range of hosts worldwide such as cereals, coffee and legumes (Sutton, 1992; Bailey & Jeger, 1992; Hyde *et al.*, 2009). Teleomorph of the fungus is in the genus *Glomerella* of the order Glomerellales of the class Sordariomycetes (Cannon *et al.*, 2012). Disease has been reported from many lentil producing countries; Canada, USA, Syria, and Brazil (Lindbeck & Ford, 2005). It was also suspected that *C. lentis* is present in Pakistan but accurate data was lacking.

Anthrachnose becomes visible in the field in patches. Symptoms on lentil are sunken necrotic lesions on leaves, stems, flowers and pods (Buchwaldt *et al.*, 1992). Lentil anthracnose is one of the most important constraints for production of lentils in Canada and can cause 100% yield loss in worst scenarios where short crop rotation, frequent rainfall and high humidity prevail (Buchwaldt *et al.*, 1992). *C. lentis* is not highly seed-borne and it is rare to find seed with more than 3% infection even when it comes from severely infested crops (Morrall, 1997). Pathogen survives between growing seasons primarily on infected stubbles. However, microsclerotia, which are formed within stem lesions, can persist freely in soil and remain viable for up to four years. Infection is initiated within the crop by infected stubbles or microsclerotia coming into contact with lentil seedlings. Controlling lentil anthracnose through cultural practices is the most commonly used strategy. Disease is best controlled through host plant resistance. Therefore, the most economical, feasible and suitable mean of controlling anthracnose is use of resistant cultivars (Bayaa *et al.*, 1997).

There are very limited resistance sources and control options available (Khola *et al.*, 2015). No detailed data on the presence and impact of lentil anthracnose in Pakistan was available. There was a research gap with regards to prevalence and incidence of lentil anthracnose in major lentil growing areas. Hence, an extensive disease survey was planned to work out scenario of this disease in major lentil growing districts of Punjab province of Pakistan.

Materials and Methods

Study area for survey of lentil anthracnose: Farmers fields located in major lentil growing districts of Punjab; Rawalpindi, Chakwal, Attock, Jhelum, Gujrat, Sialkot, Narowal, Bhakkar, Layyah, Muzaffargarh, Khushab, Mianwali and Jhang were surveyed during 2014 and 2015 crop season for assessment of prevalence, incidence and

severity of anthracnose. Detail on surveyed districts and their important areas/locations visited is presented in Table 1; Fig. 1.

Two-three anthracnose samples depending upon farm size were collected on the basis of lentil anthracnose symptoms. This involved random sampling in the infected field by using a 1m² quadrat five times. Owing to sporadic and dispersed lentil crop in respective districts; all fields along the roads were surveyed. For confirmation of the anthracnose pathogen, samples were brought to Fungal Plant Pathology laboratory of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, for identification. For disease assessment, total plants and lentil anthracnose infected plants in 1m² were calculated and disease prevalence (DP), disease incidence (DI) and disease severity (DS) were calculated by using the following formulae/scale.

Table 1. Geographical description of areas surveyed for lentil anthracnose in Punjab for 2014 and 2015.

Sr. #	District	Latitude/longitude	Location (s) surveyed for lentil anthracnose
1.	Rawalpindi	33°59'N, 73°04'E	Syed Kasran
2.	Chakwal	32°93'N, 72°85'E	Barani Agriculture Research Institute, Talagang, Lava, Bhaun, Sehgal Abad
3.	Attock	33°76'N, 72°36'E	Fateh Jang, Dhulian, Khaur
4.	Jhelum	32°94'N, 73°72'E	Sohawa
5.	Gujrat	32°57'N, 74°07'E	Lambray, Dolat Nagar, Bhadder, Jalalpur Jattan
6.	Sialkot	32°49'N, 74°53'E	Pasrur Road, Pasrur, Chowinda
7.	Narowal	32°09'N, 74°87'E	Zafarwal, Muridkay Road, Kot Nainan
8.	Mianwali	32°57'N, 71°56'E	Chashma Road, Piplan
9.	Khushab	32°30'N, 72°34'E	Noor Pur Thal, Muzaffargarh Road, Qadir Pur, Adhi Kot, Rangpur Baghoor
10.	Jhang	31°26'N, 72°31'E	Jhang - Khushab Road
11.	Bhakkar	31°62'N, 71°06'E	Arid Zone Research Institute, Kloor Kot, Mankera, Darya Khan
12.	Layyah	30°96'N, 70°93'E	Agronomy Research Station, Karor Chaubara, Kot Lal Esan
13.	Muzaffargarh	30°07'N, 71°18'E	Layyah Road, Kot Addu

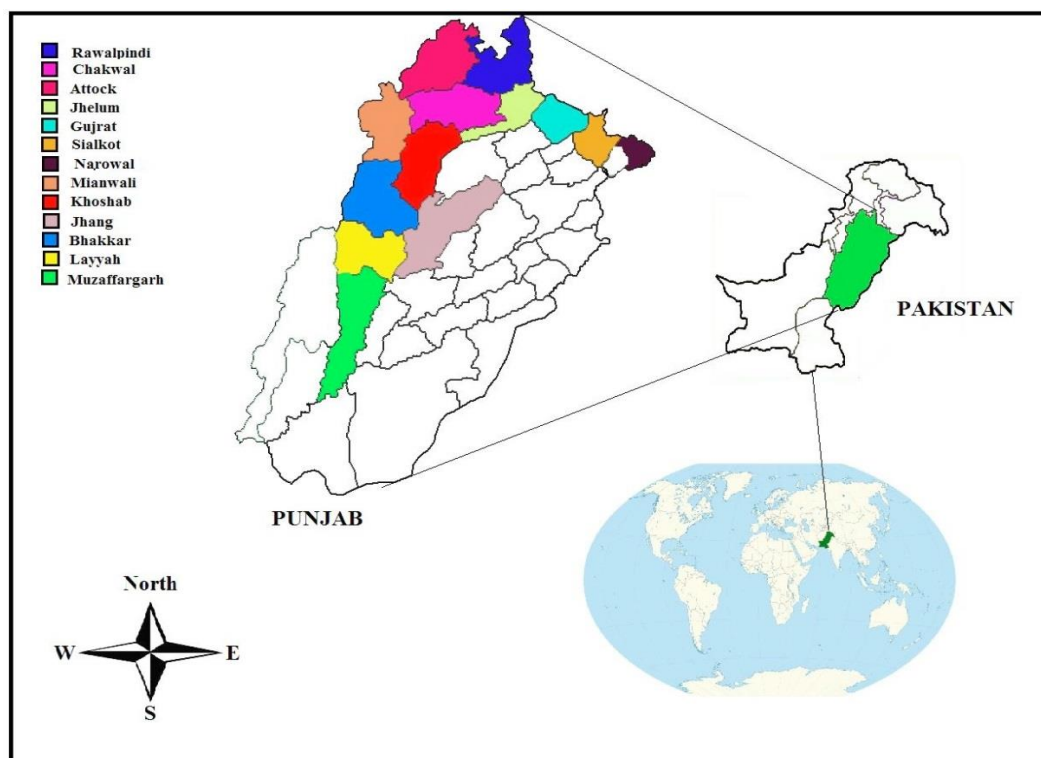


Fig. 1. Lentil growing districts of the Punjab province of Pakistan surveyed during crop season of 2014 and 2015 for assessment of anthracnose.

$$DP (\%) = \frac{\text{Number of locations showing anthracnose}}{\text{Total number of locations surveyed}} \times 100$$

$$DI (\%) = \frac{\text{Number of anthracnose infected plants}}{\text{Total number of plants}} \times 100$$

Disease severity was assessed by using 1-9 rating scale of Tullu *et al.* (2006); where rating 1= No lesions (Highly resistant) 3 = Few small superficial lesions (Resistant), 5 = Few deep lesions, mostly superficial, no stem girdling (Moderately resistant), 7 = Several deep stem lesions, partial wilting (Susceptible), and 9 = Deep penetrating lesions, stem girdling, wilting coalesce (Highly Susceptible).

Employing above formulae/scale; Lentil anthracnose prevalence, incidence and severity in each sample/ field/ location was calculated and, consequently districts mean incidence and severity were worked out. Anthracnose distribution record of the various districts of Punjab was developed.

Results

Lentil anthracnose survey and collection of samples:

Anthracnose survey was conducted during a span of two years; 2014 and 2015 crop season. As a result, 162 diseased samples were collected from the private lentil farms and government research institutes/stations scattered in 66 locations of 37 areas among 13 districts of the province of Punjab i.e. Rawalpindi, Chakwal, Attock, Jhelum, Gujrat, Sialkot, Narowal, Mianwali, Khushab, Jhang, Bhakkar, Layyah and Muzaffargarh. Year-wise, 80 anthracnose samples were collected during 2014 and 82 samples in 2015 lentil crop (Tables 2 and 3).

Anthracnose prevalence, incidence and severity:

During lentil survey, anthracnose was found in 100% areas/localities of various districts visited. At all the places, lentil plants manifested typical anthracnose symptoms (Figs. 2 and 3), however disease incidence (DI) and disease severity (DS) varied considerably in surveyed areas/localities of the districts during thisperiod (Table 2). At individual locations, the maximum mean DI 85% and 84 % in 2014 and 2015, respectively, was observed in Noor Pur Thal (district Khushab) followed by 82% (2014) in Adhi Kot (Khushab); 82 % (2015) in Pasrur (district Sialkot); 81% (2015) in Kot Nainan (district Narowal) and 80% (2014) in Pasrur (Sialkot). On the other hand, the minimum mean DI of 5% each during 2014 and 2015, respectively, was noted in Bhadder (district Gujrat) followed by 15% (2015) and 16% (2014) in Dolat Nagar (Gujrat); 19% (2014) in Fateh Jang (district Attock) and 20 % each in Lambray (Gujrat), and Jhang - Khushab Road (district Jhang) during 2014 and 2015, respectively. In Dhulian and Fateh Jang (Attock) DI of 21% each was noticed during 2014 and 2015, respectively. At rest of the locations, DI ranged between 22% (Jhang - Khushab Road) and 78% (Kot Nainan) during the two years.

Correspondingly, disease severity too, varied greatly at individual locations during the two years survey (Table 2). The highest mean DS 9; during the both years was witnessed at Chowinda (Sialkot), Kot Nainan (Narowal) and Noor Pur Thal (Khushab) followed by DS 9 in Adhi Kot (Khushab) during 2014 and DS 8 in Chaubara (District Layyah) and Kot Addu (district Muzaffargarh)

during both the years. The lowest mean DS 2 (during both years) was observed at Fateh Jang and Dhulian (Attock), Sohawa (Jhelum), Dolat Nagar, Bhadder (Gujrat), Jhang-Khushab Road (Jhang), Darya Khan (Bhakkar) and Karor Lal Esan (Layyah) followed by DS 3 in Sehgalabad (district Chakwal), Lambray and Jalal Pur Jattan (Gujrat) during 2014 and Chasma (district Mianwali) in 2015. At majority of locations, DS 4 to 5 was observed.

Year-wise, the maximum districts mean DI 69% (2014) and 67% (2015) along with mean DS 7 during both years was found in district Khushab (Figs. 4 and 5). The next districts in descending lentil anthracnose intensity were Sialkot (DI 62% in 2014 & 64% in 2015; and DS 7 during both the years) followed by Muzaffargarh (DI 59% in 2014 & 54% in 2015 and DS 6 during both the years) and Narowal (DI 55% in 2014 and 56% in 2015 and DS 6 during both the years). On the contrary, the minimum districts mean DI 20% in 2015 and 22% in 2014 together with DS 3 in both the years was noticed in Jhang followed by Gujrat DI 19 % and DS 3 in 2014 and DI 28% and DS 4 in 2015. Similar DI and DS were noticed in district Mianwali and Attock (DI 33% during 2014 and 36% during 2015 along with DS 4 during both the years).

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Table 2. Prevalence, incidence and severity of lentil anthracnose in various areas/locations of the districts of Punjab (crop season; 2014 and 2015).

Sr.	District	Area(s)/ location(s)	Total location(s) surveyed	Total farms surveyed		Prevalence (%)			Incidence (%)			Severity (1-9 rating)			
				2014	2015	Total	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean
1.	Rawalpindi	Syed Kasran	1	2	2	4	100	100	100	38	47	43	4	5	5
		Barani Agri. Res. Inst.	1	2	2	4	100	100	100	66	72	69	7	7	7
		Talagang	1	2	3	5	100	100	100	47	45	46	5	5	5
2.	Chakwal	Lava	1	2	2	4	100	100	100	55	48	52	6	5	6
		Bhaun	1	2	2	4	100	100	100	48	41	45	5	4	5
		Sehgal Abad	2	3	3	6	100	100	100	31	39	35	3	4	5
		Fateh Jang	3	2	4	6	100	100	100	19	21	20	3	3	3
3.	Attock	Dhulian	1	1	1	2	100	100	100	21	36	29	3	3	4
		Khaur	1	2	1	3	100	100	100	59	50	55	6	5	6
		Sohawa	1	2	1	3	100	100	100	31	39	35	3	3	4
4.	Jhelum	Lambray	1	1	1	2	100	100	100	20	40	30	3	4	4
		Dolat Nagar	1	2	2	4	100	100	100	16	15	16	3	3	3
5.	Gujrat	Bhadder	1	1	2	3	100	100	100	5	5	5	3	3	2
		Jalalpur Jattan	1	2	2	4	100	100	100	36	51	44	3	5	5
		Pasur Road	1	2	2	4	100	100	100	42	39	41	4	4	5
6.	Sialkot	Pasur	2	2	2	4	100	100	100	80	82	81	7	7	8
		Chowinda	2	3	3	6	100	100	100	65	70	68	9	9	7
		Zafarwal	3	3	3	6	100	100	100	40	38	39	4	4	4
7.	Narowal	Muridkay Rd	4	2	2	6	100	100	100	48	50	49	5	5	5
		Kot Nainan	4	5	5	10	100	100	100	78	81	80	9	9	8
		Chashma	2	2	2	4	100	100	100	40	39	40	4	3	4
8.	Mianwali	Piplan	2	3	3	6	100	100	100	26	32	29	4	4	4
		Noor Pur Thal	4	5	5	10	100	100	100	85	84	85	9	9	8
		Muzaffargarh Road	2	2	2	4	100	100	100	60	67	64	6	7	7
9	Khushab	Qadir Pur	1	1	2	3	100	100	100	46	35	41	4	4	5
		Adhi Kot	2	3	3	6	100	100	100	82	77	80	9	8	8
		Rangpur Baghoor	3	3	3	6	100	100	100	71	74	73	7	7	7
10	Jhang	Jhang-Khushab Road	1	1	1	2	100	100	100	22	20	21	3	3	3
		Arid Zone Res. Inst.	1	2	1	3	100	100	100	41	42	42	4	5	5
		Kloor Kot	2	2	2	4	100	100	100	75	66	71	8	7	8
11	Bhakkar	Mankera	1	1	2	3	100	100	100	36	40	38	4	4	5
		Darya Khan	2	1	1	2	100	100	100	23	27	25	3	3	4
		Chaubara	3	2	2	4	100	100	100	76	75	76	8	8	8
12	Layyah	Agro. Res. Sta. Karor	1	1	1	2	100	100	100	41	42	42	4	5	5
		Karor Lal Esan	2	2	2	4	100	100	100	27	29	28	3	3	4
		Layyah Rd.	2	2	2	4	100	100	100	42	37	40	4	4	5
13	Muzaffargarh	Kot Addu	2	2	3	5	100	100	100	76	71	74	8	8	8



Fig. 2. A typical lentil anthracnose field in the suburbs of district Sialkot manifesting disease patches during 2015 crop season.



Fig. 3. Sunken necrotic lesions caused by *C. lentis* on lentil plant.

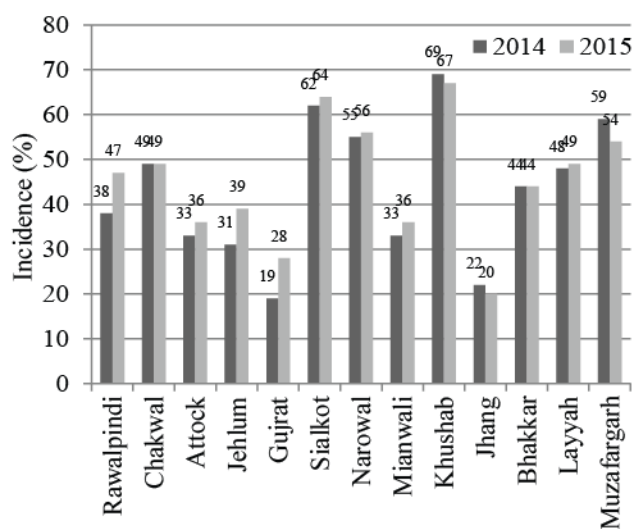


Fig. 4. Year-wise; mean incidence percentage of lentil anthracnose in various districts of the Punjab.

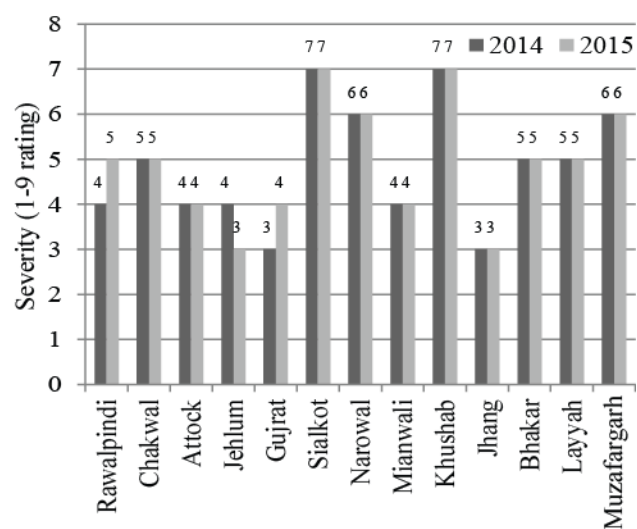


Fig. 5. Year-wise; mean severity (1-9 rating) of lentil anthracnose in various districts of the Punjab.

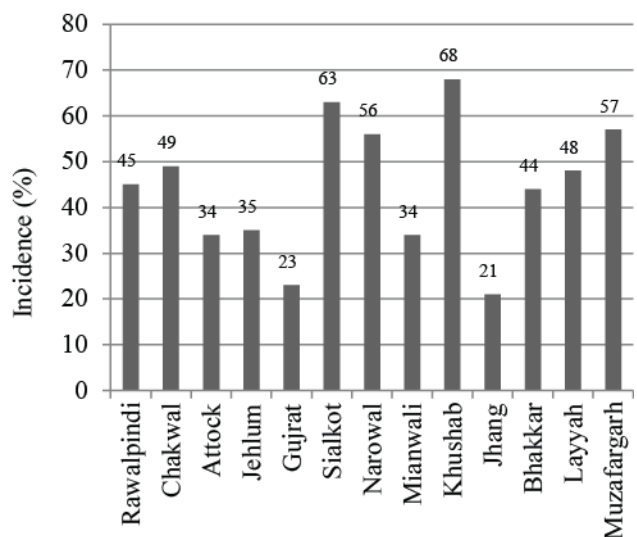


Fig. 6. District-wise; mean incidence percentage in thirteen districts of the Punjab (2 years mean; crop season 2014 and 2015).

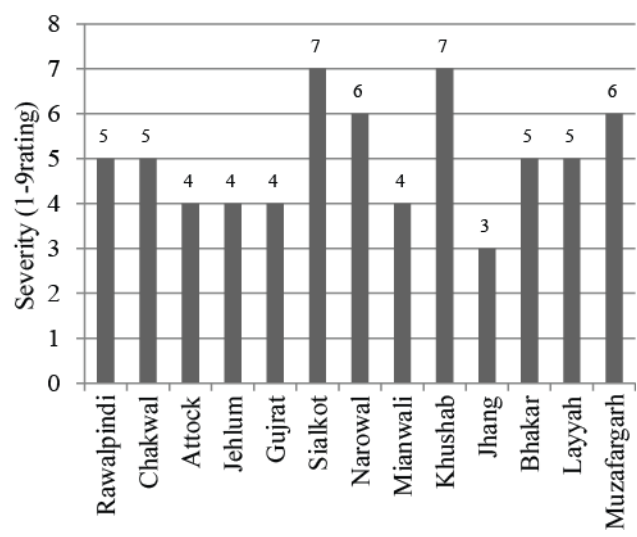


Fig. 7. District-wise; mean severity (1-9 rating) of lentil anthracnose in thirteen districts of the Punjab (2 years mean; crop season 2014 and 2015).

Table 3. District-wise; mean prevalence, incidence and severity of lentil anthracnose in the thirteen districts of Punjab (2 years mean; crop season 2014 and 2015).

Sr.	District	Total area(s) surveyed	Total location(s) surveyed	Total farms surveyed	Prevalence (%)	Incidence (%)	Severity (1-9 rating)
1.	Rawalpindi	1	1	4	100	43	5
2.	Chakwal	5	6	23	100	49	5
3.	Attock	3	5	11	100	35	4
4.	Jhelum	1	1	3	100	35	4
5.	Gujrat	4	4	13	100	24	4
6.	Sialkot	3	5	14	100	63	7
7.	Narowal	3	11	22	100	56	6
8.	Mianwali	2	4	10	100	35	4
9.	Khushab	5	12	29	100	68	7
10.	Jhang	1	1	2	100	21	3
11.	Bhakkar	4	6	12	100	44	5
12.	Layyah	3	6	10	100	49	5
13.	Muzaffargarh	2	4	9	100	57	6
Total		37	66		162 (2014=80 2015=82)		

Year-wise, the maximum districts mean DI 69% (2014) and 67% (2015) along with mean DS 7 during both years was found in district Khushab (Figs. 4 and 5). The next districts in descending lentil anthracnose intensity were Sialkot (DI 62% in 2014 & 64% in 2015; and DS 7 during both the years) followed by Muzaffargarh (DI 59% in 2014 & 54% in 2015 and DS 6 during both the years) and Narowal (DI 55% in 2014 and 56% in 2015 and DS 6 during both the years). On the contrary, the minimum districts mean DI 20% in 2015 and 22% in 2014 together with DS 3 in both the years was noticed in Jhang followed by Gujrat DI 19 % and DS 3 in 2014 and DI 28% and DS 4 in 2015. Similar DI and DS were noticed in district Mianwali and Attock (DI 33% during 2014 and 36% during 2015 along with DS 4 during both the years).

Based on the two years districts mean data, the highest DI 68% and DS 7 was found at district Khushab followed by Sialkot DI 63% and DS 7; Muzaffargarh DI 57% & DS 6; and Narowal DI 56% and DS 6 (Table 2; Figs. 6 and 7). Whereas, the lowest DI 21% and DS 3 was found at Jhang followed by Gujrat DI 24%; Attock DI 35%; and Jhelum DI 35%. It is interesting to note that similar DS i.e. 4 was observed in all the three districts. Rest of the districts showed almost an intermediate DI and DS.

Discussion

In Pakistan, little attention has been given to the pathology of lentil, therefore to bridge this gap an extensive and methodical two years survey was conducted in the major lentil producing districts of the Punjab; Rawalpindi, Chakwal, Attock, Jhelum, Gujrat, Sialkot, Narowal and Thal (Khushab, Mianwali, Bhakkar, Layyah, Muzaffargarh and Jhang districts are collectively known as Thal or sometimes Thal desert). It is located between the rivers Jhelum and Sindh near the Potohar Plateau. Province of the Punjab was selected for lentil anthracnose appraisal because more than 75% of the country's lentils are planted here; thereby it contributes a major share to the country's lentil production. Environmental conditions of this area

comprise both the extremes; cooler in the north and hot, barren in the south. At large, the temperature is hot with distinct variations in summer and winter times. Daily temperature (average) ranges from 30-10°C with an average annual rainfall from 180-580 mm among studied districts. Area is generally productive along the river valleys, although scanty deserts are present near the boundary touching Rajasthan and the Suleiman Range area.

Districts-wise, significant variations in anthracnose incidence and severity were noted, perhaps owing to various factors like environmental conditions and the cultivars grown there. Consequently, by reason of the fact that disease is more temperature-dependent (around 23 ± 2°C), which is commonly observed during the month of March in the Punjab, therefore the lentil crop grown here revealed vulnerability to anthracnose. Mostly, cvs Masoor-85 and Masoor-2002 were the dominating cvs growing in the districts Khushab, Sialkot, Muzaffargarh and Narowal at the time of survey; DI 68% & DS 7; DI 63% & DS 6; DI 57% & DS 7; and DI 56% & DS 6, respectively, were found in these districts. It appears cvs Masoor-85 and Masoor-2002 found susceptible towards lentil anthracnose in the presence of availability of favorable disease conditions like warm and soggy, which were witnessed there. According to Bailey *et al.* (2003), warm and damp environmental conditions favor occurrence and progress of lentil anthracnose. Similarly, (Chongo & Bernier, 2000) reported 100% DI at the four day (16, 20, 24, and 28°C) and night temperatures (12, 16, 20, and 24°C) in the susceptible lentil cv. Eston using a single *C. lentis* isolate under greenhouse conditions. However, between 33 and 100% DI was among the five partially resistant lentil genotypes viz. PI 299331, PI 320937, PI 345629, 458-57, and cv. Indianhead. In another study, Chongo & Bernier (2000a) reported a temperature of 20-24°C for optimal disease development. On the other hand, the lowest DI and DS were observed in Jhang (DI 21% and DS 3), Gujrat (DI 24% and DS 4) and Attock (DI 35% and DS 4). This anthracnose situation may be due to the fact that there was not enough moisture in the environment during disease survey as microsclerotia of *C. lentis* are rain-splashed from the soil

to leaflets and stems of lentil. From this the secondary conidial inocula is generated, which further spread to surrounding plants, especially during rains thus ending up in recurring pathogen infection cycles (Bailey *et al.*, 2003). Between fields, blowing winds can carry microsclerotia, thus playing an important role in the subsequent spread of anthracnose (Buchwaldt *et al.*, 1996; Bailey *et al.*, 2003). So absence of such conditions in these districts might be the cause of less disease there.

A substantial variation in DI and DS shows that lentil anthracnose is established in the areas surveyed but the differences in disease intensity may be due to different levels of susceptibility/resistance in the cvs growing there, that's why cvs are behaving contrarily towards anthracnose pathogen. To sum up, lentil anthracnose is prevalent in lentil growing areas of the Punjab and demands attention from concerned quarters as it may break out any moment in the epidemic form provided disease conditions are favorable. Similarly, relevant international literature specifies *C. lentis* as an important and devastating pathogen of lentils in many areas of the world e.g. North America, where it is a grave threat to lentil production (Morrall, 1988; Anderson *et al.*, 2000; Armstrong & Banniza, 2006; Shaikh *et al.*, 2013).

Conclusion

This comprehensive survey shows an up-to-date status of lentil anthracnose disease in the Punjab, Pakistan. It makes farmers and researchers aware that lentil anthracnose exists in the crop growing areas/districts of Punjab, however its incidence and severity varies highlighting the need for regular and targeted surveillance surveys by the concerned quarters. Consequently, prevailing lentil anthracnose scenario should not be ignored as favorable disease conditions for lentil anthracnose can pose a serious threat to lentils, hence demands effective management. Based on the current findings; scientists may take up further investigations like biology and integrated disease management of *C. lentis* to keep lentil anthracnose under control to evade the crop losses.

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