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ECOLOGICAL AND MORPHOLOGICAL PERSPECTIVES OF ROOT ROT PATHOGENS OF PEANUT (*ARACHIS HYPOGEA* L.) IN POTHWAR REGION OF PUNJAB, PAKISTAN

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Abstract

Soil borne diseases have been known as one of the significant limiting factors in peanut production. In Pakistan, little research has been done on peanut (*Arachis hypogea* L.) root rot. The present survey was carried out for the assessment of disease incidence and isolation of phytopathogenic fungi causing root rot of peanut in seven tehsils of three districts of Pothwar regions viz., Chakwal (Chakwal, Talagang), Rawalpindi (Gujar khan), Attock (Attock, Jand, Pindi gheb, *Fateh Jang)*. Results revealed that root rot of peanut prevalence was 61.25% in surveyed area. "Two hundred and sixty-five (265)" infected roots samples were collected from 480 farmer's fields of 79 villages during two years. During survey, it was observed that the root rot susceptible cultivar No.334 was grown in more than 70% cultivated area of peanut. The highest disease incidence was 14.2% and 15.9% in tehsil Attock, while the lowest was 8.0% and 9.6% in tehsil Gujarkhan during 2019 and 2020 respectively. A total of 104 fungal isolates were obtained from the peanut cultivation areas were *Athelia rolfsii* (49%) followed by *Fusarium fujikuroi* (29.8%) and *Aspergillus terreus* (21.2%). *Athelia rolfsii (*CHGR2a*)* had the utmost virulence followed by *Fusarium fujikuroi* (CHGRF5) and *Aspergillus terreus* (TGGR7). The pathogenicity test on peanut cultivar No.334 revealed that all the 104 isolates were pathogenic but among them 06 isolates (PGGR6, CHGR2a, ATGR3, PGGR12, TGGR7 and CHGRF5 were highly pathogenic. Disease incidence and severity values of 104 isolates varied between 15.33% to 93.3% and 13.53% to 89.53%. The disease incidence values of *Athelia rolfsii, Fusarium fujikuroi* and *Aspergillus terreus* ranged from 15.53% - 93.3%, 24.4%-88.9% and 24.4% - 84.4% while disease severity values ranged from 13.53% - 89.53%, 19.7% - 84.2% and 20.6% - 79.7% respectively.

Key words: Peanut, Survey, Root rot, *Fusarium, Sclerotium*, *Aspergillus*, Incidence, Pothwar Region.

Introduction

Peanut (*Arachis hypogaea* L) is one of the top 15 world's most significant food crops. It is a very important edible leguminous oilseed crop. It was first cultivated in South America 7500 years ago and is commercially cultivated among 40°N to 40°S the latitude and upto 1,000 meters of altitude. In the first century, it spread to Mexico and further to North America, Africa, China, and India (Pattee & Young, 1982). More than 50% of the world's total peanuts are produced by two countries i.e., China and India. During marketing year 2020/21, global gross production of peanut was 50.17 million metric tons. China was the world's largest peanut producer producing 17.99 million metric tons and India was the next with 6.70 million metric tons (Anon., 2022). To cover the gap into demand and supply, introducing high yielding varieties and developing better agronomic practices is the best alternative for good production.

In Pakistan Pothwar region, it is cultivated as a cash crop (Ali *et al*., 2002). Its province wise cultivation is distributed as, 85% area in Punjab, 10% in KPK and 5 % in Sind (Asad *et al*., 2017). In Pakistan during 2019-20, total peanut cultivated area was 102.95 thousand hectares which province wise distribution was Punjab 95.85 thousand hectares, Sindh 0.599 thousand hectares and Khyber Pakhtunkhwa 6.50 thousand hectares (Anon., 2021).

During 2019-20, total peanut production in Pakistan was 94.52 thousand tonnes, with share of Punjab 86.42 thousand tonnes, Sindh 1.20 thousand tonnes and Khyber Pakhtunkhwa 6.90 thousand tonnes. Similarly in the same year average yield of peanut in Pakistan was 917.85kg/hectares with provinces as Punjab 902.0 kg/hectares, Sindh 1998.72 kg/hectares and Khyber Pakhtunkhwa 1061.11kg/hectares (Anon., 2021).

In rainfed areas it is known as a good crop for crop rotation and a drought tolerant crop. As a leguminous crop it fixes atmospheric nitrogen that maintains soil productivity. It is also an energy rich crop that contains 25 to 30% proteins, 5 to 15% carbohydrate and 50 to 55% oil.

A variety of stresses affect peanut production from planting to storage. Among these, diseases are the major threat. The soil-borne fungal pathogens effects are mostly obscured due to their short-range dispersal capacity (Termorshuizen, 2017). Each year disease causes a major loss in the peanut production. Peanut production losses caused by mostly soil borne plant pathogens which create the problems in diseases diagnosis that shows similarity in symptoms. Various fungi and nematodes that cause soil borne diseases of peanut infect one or more parts of the plant at or under the soil surface (Damicone, 2017). The pathogens are responsible for various disease symptoms, like, root rot, charcoal rot, seedling blight, dry rot, pod rot, and seed rot, and also causes significant yield losses in peanut (Kumar *et al*., 2020). In July 2021, a root rot disease was detected on peanut in Laiwu (36.22°N, 117.67°E), Shandong Province, China. The disease incidence was approximately 35%. The symptoms of the disease comprised of root rot, vessels with a brown to dark brown discoloration, and progressive yellowing and wilting of leaves from the base leading to whole plant death (Li *et al*., 2023).

Peanut root rot disease affects the production and quality of peanut crop, it is a seed-borne disease caused by a complex of pathogens. The disease progresses in different forms like collar rot (*Aspergillus*), stem-rot (*Sclerotium*)

and root-rot (*Rhizoctonia or Fusarium*). About 30-55% yield loss was recorded in Pakistan, by black symptoms on roots and stem affecting the vascular system, resulting in complete wilting and plant death. *F. solani* (Mart) Sacc., which causes peanut root rot is economically important and most destructive soil borne fungus in peanut growing areas (Semangun, 1993). *Fusarium fujikuroi* has been reported to cause root rot and wilt on soybean (Pedrozo *et al*., 2015; Zhao *et al*., 2020). *F. fujikuroi* can infect plants through root tips, stem base, seeds, but not the leaf blade and stem (Sun, 1975). *Aspergillus terreus* causes direct damage to the important crops such as potato, rice, sugar cane, wheat, maize, and soybean through overall plant contamination and seed (Maryam *et al*., 2015). A new pathogen *Aspergillus terreus* Thom, causes foliar blight of potato (Louis *et al*., 2013) whereas in peanut growing regions of the world root rot causes almost 95% disease incidence. In Attock the highest mean root rot disease incidence was observed at 63%, while in Khushab no disease was found (Zaman & Shakil, 2012).

In the recent years root rot of peanut caused considerable yield losses in peanut cultivated areas of Pothwar region of Punjab. Surveys indicated that soil borne diseases were the major cause for poor yield of peanut in this area. Therefore the present study was carried out during two successive years (2019 and 2020), with the objectives to evaluate the status of the (i) root rot incidence, (ii) to isolate the local pathogenic agents of root rot of peanut and (iii) to test the pathogenicity of these isolated pathogens.

Materials and Method

Survey: A survey was conducted in peanut growing area to estimate the incidence and prevalence of root rot disease, during the months of August to October in cropping seasons 2019 and 2020 in three districts, Chakwal (33°06'N; 72°64'E), Rawalpindi (33°37'N; 73°2'E) and Attock (33°91'N; 72°58'E) of Pothwar region of Punjab, Pakistan. In this survey, information on crop disease and socioeconomic aspects were noticed on a structured questionnaire. In each district, 7-12 villages of one Tehsil were visited. Three fields were randomly selected in a village at proper intervals of between 3 - 4 km and village to village distance was between 13-15km along the main and frontage roads. The disease incidence data was calculated in every field from five different locations by an open quadrat in a square meter area (1×1 m^2). The basic land unit in this region is 1648 m^2 or 0.17 hectare for a field which was used as disease survey and sampling unit. Disease incidence data was assessed in 474 farmer's fields (237/year) from total 79 selected peanut growing villages. In tehsil, Chakwal (36), Talagang (30), Attock (36), PindiGheb (36), Jand (30), Faith Jang (24) and Gujarkhan (45) fields were surveyed during each year. To assess the disease incidence, as an average ten plants in each quadrat were selected. Per cent disease incidence was calculated by using the equation described by (Nutter *et al*., 1991).

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Percent disease severity was assessed by using 0 – 4 scale slightly modified from (Ledingham *et al*., 1973), 0 = healthy plant, 1 = Yellowing + curvature ¼ leaflets (< 25%), 2 = Yellowing + curvature ½ leaflets (25–50%), 3 = Yellowing + curvature¾ leaflets and brown necrotic spot (~75%), 4 = death of the whole plant. Percent disease severity index was calculated by converting the disease severity rating based on above scale values by using equation as suggested by (Wheeler,1969).

The final disease incidence and severity data was explained as the average disease in a village (location) by taking the average of three fields in each village. Disease prevalence was calculated based on locations (fields) representing root rot problems. Using a Global Positioning System (GPS) device, coordinates were noticed at each data collecting and sampling site (Table 1). The numbering of surveyed fields per district was dependent on the accessibility of peanut area during survey.

The elevation of the survey area ranged from 317 to 677 meters above sea level. Average annual rainfall was 277.3 mm in 2019 and 309.4 mm in 2020. Average temperature ranged from 15◦C to 37◦C in 2019 and 12◦C to 38◦C in 2020 (Anon., 2020a). It was observed that, a highly susceptible peanut local cultivar (No.334) was cultivated on about more than 70% surveyed area.

Soil characteristics: Physical and chemical properties of the soil collected during survey were also checked. The texture of the soil was sandy loam to loam and in some places silt to clay loam (Malik *et al*., 1984), pH level was ranged from 7.44 to 8.2 (Schofield & Taylor, 1955). The organic matter was from 0.46-1.17% in soil (Nelson & Sommers, 1982). Phosphorous (Olsen & Sommers, 1982) and Potash (Helmke & Sparks, 1996) were 4.8-12.3 and 120-180 mg/kg respectively. The micronutrients (Iron, Zinc, Manganese, Copper, Sulphur and Boran) concentration was 0.26-1.17, 0.12-0.85, 2.2-5.7, 0.23-1.19, 5.8-8.5 and 0.14-0.45 mg kg^{-1}, respectively.

Sample collection: A total of 265 samples collected in two years, showed the evident symptoms of root rot collected from different locations of 79 villages in Pothwar region of Punjab. Plants showing disease symptoms were uprooted and samples were cutted 5 cm above the crown area. The infected root parts of the samples were placed in polythene bags and brought to the laboratory.

Isolation of pathogens: Diseased peanut plant roots from each field showing distinctive root rot symptoms were cut into small pieces and mixed. Isolations of fungi were done from 4-5 symptomatic plants per field. Small pieces of diseased parts were sterilized by soaking in 1% sodium hypochlorite (NaOCl) solution for 2 minutes.

After washing 3 times in uncontaminated distilled water, samples were dried on filter paper. Four different media were used for isolation of pathogens, such as Potato Dextrose Agar (PDA), Czapek's agar (CZA), Rose Bengal Agar (RBA) and Malt Extract Agar (MEA). Approximately 30 root segments (~3-4 cm) per sample were imprinted on each media (6 petri plates) and incubated at 25±2ºC for 6-7 days. The purity of each fungal culture was assessed by examination of colony morphology. For purification a single spore from each developed fungus was placed into PDA medium. Purified culture inoculum was shifted to PDA slants and incubated at 25ºC. In this way, a total of 104 isolates were obtained from 265 root samples. The isolates were kept in a refrigerator at 4ºC and stored at –20ºC containing 30% glycerol for long storage.

Table 1. Field survey conducted in six Tehsils of Pothwar region of Punjab, Pakistan (2019-20).

Sr. No.	Name of tehsils	Name of Villages	Coordinates	Elevation (m)	Soil type	Cultivar	Surveyed fields
1.	Gujarkhan	Sarahadnay,U.C Manghot, Gujarkhan	N 33.25155, E 073.14813	553	Loam	Unknown, No.334	3
2.		Village Dhok Sakrila, Gujarkhan	N 33.26595, E 073.15056	553	Loam	Unknown	3
3.		Village Daulat, Gujarkhan	N 33.27716, E 073.15756	554	Sandy loam	Golden, Bari-16	3
4.		Chak Maradi,U.C Sukho, Gujarkhan	N 33.23626, E 073.17539	532	Clay loam	No.334	3
5.		Village jatli, Dhok Karmi Gujarkhan	N 33.19738, E 073.09371	567	Clay loam	Unknown, No.334	3
6.		Village Phimbl, Jhangi Phairu, Gujarkhan	N 33.17851, E 073.08079	521	Sandy loam	Bari2000	3
7.		Village Phimbl, Jhangi Phairu, Gujarkhan	N 33.17350, E 073.04875	532	Loam	Bari-11, unknown	3
8.		Village Rama, Jhangi Phairu, Gujarkhan	N 33.17848, E 073.08080	491	Loam	No.334	3
9.		Village Sukho, P.O Sukho, Gujarkhan	N 33.25155, E 073.19523	510	Silt loam	No.334	3
10.		Bhakkar ada, U.C Sukho, Gujarkhan	N 33.28112, E 073.17156	557	Loam	Bari-16	3
11.		Bhakkar ada, U.C Sukho, Gujarkhan	N 33.16962, E 073.14974	557	Sandy loam	Unknown, No.334	3
12.		Village Natha chattar U.C Doltla, Gujarkhan	N 33.17336, E 073.14198	497	Loam	Bari-00, unknown	3
13.		Village Natha chattar U.C Doltla, Gujarkhan	N 33.16921, E 073.14182	555	Sandy loam	No.334, Golden	3
14.		Village Qatbal, Kanyal Bajrana, Gujarkhan	N 33.15048, E 073.18618	541	Sandy loam	Unknown, No.334	3
15.		Thakra more, Bardiana, Gujarkhan	N33.10579, E 073.19393	547	Loam	Golden	3
16.	Attock	U.C Haji Shah, China Chok, Attock	N 33.81955, E 072.34100	378	Loam	No.334, Bari-11	3
17.		Groundnut Research Station, Attock	N 33.79475, E 072.31142	365	Loam	No-334	3
18.		Moza shakardara, U.C Shen bag, Attock	N 33.79375, E 072.31443	365	Sandy loam	No-334, Golden	3
19.		Ghazi brotha, U.C Romban, Attock	N 33.81956, E 72.39049	378	Sandy loam	No-334	3
20.		U.C Kamra, Urtakpur, Attock	N33.855881, E 072.39052	379	Sandy loam	No-334	3
21.		Kamra Kalan. Attock	N 33.82193, E 072.38069	380	Loam	No-334	3
22.		U.C Dharnal, Moza Ddhair Kamran, Attock	N 33.80700, E 72.41811	380	Loam	Golden	3
23.		U.C Mirza, Boora, Attock	N 33.80545, E 072.42039	381	Loam	No-334	3
24.		U.C Kisran, Karma Attock	N33.811111, E 072.41241	374	Loam	Unkown, No-334	3
25.		U.C Kamra, Village Graa, Attock	N 33.82764, E 072.38392	364	Loam	Bari-16	3
26.		U.C Kamra, village Kisra, Attock	N 33.85911, E 072.39229	317	Loam	No-334, golden	3
27.		Village Mirza, U.C Mirza, Attock	N 33.82763, E 072.33494	367	Loam	No-334	3
28.	Jand	Nai Abadi,U.C, Jand	N 33.41151, E 071.98576	355	Loam	No-334	3
29.		Village Chura Sharif, U.C Langar, Jand	N33.453074, E 072.11003	354	Loam	Bari-16	3
30.		Village Saghri, Jand	N33.589262, E072.08542	353	Loam	No-334, Bari-11	3
31.		Kot Chaji, Jand	N 33.56291, E 072.07733	350	Sandy loam	Unknown, No.334	3
32.		Mariala, Jand	N 33.62401, E 072.09439	359	Loam	N0-334	3
33.		kot Chaji, U.C Jalwal, Jand	N 33.56375, E 072.07310	331	Sandy loam	Golden	3
34.		Sagri, U.C Sagri, Jand	N 33.60808, E 072.09407	356	Sandy loam	Unknown	3
35.		Near olive Garden Saghri, U.C Saghri, Jand	N 33.58934, E 072.08305	324	Loam	Bari-16	3
36.		Dhok Jhangi, U.C Sagi, Jand	N 33.52476, E 072.06813	338	Sandy loam	No-334, golden	3
37.		Rangli, U.C Pind Sultani, Jand	N 33.50593, E 72.13799	346	Sandy loam	Bari-11	3

Table 1. (Cont'd.).

Sr. No.	Name of tehsils	Name of Villages	Coordinates	Elevation (m)	Soil type	Cultivar	Surveyed fields
38.	Pindighabe	Thalli Karla, U.C Dandi, Pindi Gheb	N 33.35320, E 072.29293	429	Loam	No-334	3
39.		Moza Langrial, U.C Dandi, Pindi Gheb	N 33.30285, E 072.29452	381	Loam	No-334	3
40.		Thathi Karla, Dhok Ganganwali, Pindi Gheb	N 33.35146, E 072.29709	423	Loam	Bari-16	3
41.		Moza Rattarian, U.C Ikhlas, Pindi Gheb	N 33.28452, E 072.39170	411	Loam	No.334	3
42.		U.C Mianwala Moza Mianwala, Pindi Gheb	N 33.36908, E 072.29374	437	Loam	Bari-11	3
43.		Moza Khaur, U.C Khaur, Pindi Gheb	N 33.24793, E 072.43791	445	Sandy loam	Bari-16, bari2000	3
44.		U, C Garibwala Dhok Nambardar, PindiGheb	N 33.17739, E 72.27252	432	Sandy loam	Golden	3
45.		Dhok Inayat, U.C Dandi, Pindi Gheb	N 33.32924, E 072.36221	413	Loam	No-334	3
46.		Dhok Inayat, U.C Dandi, Pindi Gheb	N 33.30283, E 072.29051	383	Loam	Bari2000	3
47.		Makial, U.C Ahmdal, Pindi Gheb	N 33.27237, E 072.40283	426	Loam	No-334	3
48.		Makyal, U.C Ahmadyal, Pindi Gheb	N 33.27441, E 072.42077	427	Sandy loam	Unknown, No.334	3
49.		Khaur, Pindi Gheb	N 33.24795, E 072.43789	429	Loam	No-334	3
50.	Fatehjang	Village Khidwal U.C Jhandial, Fathajang	N 33.37977, E 072.58744	529	Loam	Bari-11	3
51.		Dhoke Bhira, Faithjang	N 33.33405, E 72.59257	524	Loam	No.334	3
52.		Village Sakhwal, U.C Dhurnal, Faithajang	N 33.34699, E 072.57721	521	Loam	Bari-16	3
53.		Village Sakhwal, U.C Dhurnal, Faithajang	N 33.37887, E 072.57212	518	Loam	N0-334	3
54.		Village Sakhwal, U.C Dhurnal, Faithajang	N 33.39582, E 72.64310	527	Loam	No-334	3
55.		Dhoke Nathiwala,Faithajang	N 33.38129, E 072.66055	536	Loam	Unkown, Bari-16	3
56.		Ali Muhammad, Fathajang	N33.416049, E072.59291	537	Loam	No-334	3
57.		Dhoke Maiki, Faithajang.	N33.407156, E072.56825	532	Loam	No.334	3
58.	Chakwal	U.C Karsal, Village Bhgwal, Blksar, Chakwal	N 33.06064, E 072.59105	421	Sandy loam	No-334	3
59.		Village Chawli, U.C Karsal, Chakwal	N 33.05125, E 072.58283	429	Sandy loam	Unknown,No.334	3
60.		Village Rupwal, U.C Chodryn, Chakwal	N 33.06687, E 072.56909	436	Sandy loam	Bari-16	3
61.		U.C Begal, Village Gha, Blksar, Chakwal	N 33.06408, E 072.63751	440	Sandy loam	No-334	3
62.		U.C Begal, Markaz Balksar, Chakwal	N 33.04580, E 072.64143	444	Sandy loam	No-334	3
63.		Village Sidhar, U.C Bhkari Kalan, Chakwal	N 32.99439, E 072.64323	502	Loam	Bari-16	3
64.		U.C & Village Bhkari Kalawn, Chakwal	N33.346860, E 072.57719	509	Sandy loam	No-334, golden	3
65.		Akwal, U.C Malik Wal, Chakwal	N 33.05126, E 072.58286	429	Sandy loam	No-334	3
66.		Budhil, U.C Budhial, Chakwal	N 33.00366, E 072.18309	411	Loam	No-334	3
67.		Malikwal, U.C Malikwal, Chakwal	N 32.91435, E 072.36552	471	Sandy loam	Bari-16	3
68.		Kotira, U.C Chingi, Chakwal	N 32.75930, E 072.37233	649	Sandy loam	Bari2000	3
69.		Chingi, U.C Chingi, Chakwal	N 32.71920, E 072.36511	677	Sandy loam	No-334, Bari11	3
70.	Talagang	Bilalabad, U.C Saghar, Talagang	N 33.00440, E 072.15735	390	Loam	unknown, Bari16	3
71.		Taman, U.C Taman, Talagang	N 33.00440, E 072.15737	392	Loam	No-334	3
72.		Patwali,U.C Badhayl, Talagang	N 33.02478, E 072.18346	400	Loam	Unknown, bari16	3
73.		Mogla, U.C Dholar, Talagang	N 32.98534, E 072.29250	431	Loam	No-334	3
74.		Sangwala U.C Badhil, Talagang	N 32.98434, E 072.23393	432	Sandy loam	Unknown, No.334	3
75.		Chokira, U.C Jasial, Talagang	N 33.94667, E 072.33671	456	Loam	No-334	3
76.		Dhok Patwari, jhatla, Talagang	N 32.86688, E 072.38327	533	Loam	Bari-16, unknown	3
77.		Khichian, U.C Jhatla, Talagang	N 32.77771, E 072.36411	593	Loam	Unknown, bari-00	3
78.		Jhatla, U.C Jhatl a, Talagang	N 32.80804, E 072.37899	606	Loam	No.334	3
79.		Dhok Jnda, U.C Thoa Mhram Khan, Talagng	N 32.76025, E 072.36819	612	Loam	No-334	3

Pathogenicity test: To measure the level of virulence, pathogenicity of the 104 fungal isolates was tested in pot experiment. Four replicated pots were employed for each fungal isolate. Peanut seedling pots with sterile non-infected soil were used as control treatment. Most susceptible peanut cultivar No.334, obtained from "Barani Agricultural Research Institute", Chakwal was used for the pathogenicity test. It is the most prominent and highly susceptible cultivar to root rot in peanut growing area of Pothwar region. Healthy and uniform sized seed of cultivar No.334 were disinfected in 1% sodium hypochlorite (NaOCl) for 2 min., followed by rinsing three times in distilled water. Rinsed seeds were dried for 12 hr. on filter paper at 24°C in laminar flow hood.

Inoculum of each isolate was prepared by taking 8 mycelium discs (5mm dia.) from the margins of 8-days old fungal culture on PDA medium. Inoculums were grown in 500 ml conical flasks having 100 gram disinfected corn meal and ground peanut shells mixture along with 30% of distilled water and were sterilized at 121°C, 15 lb pressure for 40 min. Inoculation of the pure culture of each isolate was done in these flasks under aseptic conditions and was incubated at 27 ± 1°C for 15 days (TH Abd-El-Moit, 1985).

Earthen pots of 25cm diameter were autoclaved at 121ºC for 30 min. and were filled with 5kg mixture of sterilized silt loam soil, sand and peat moss with volume ratio of (1:1:1), (Nerey *et al*., 2010). This soil mixture was sterilized with 5% formaldehyde solution @ 100 ml/kg of soil. Soil in pots was infested with one culture (15 days old) of one of the 104 test isolates @ 1% (w/w) by mixing it into the soil. Pots having soil mixed with an equivalent amount of non-pathogenic media served as control. Four replicated pots were employed for each treatment. Before sowing the seeds, pots were kept in greenhouse for 7 days with a day-time temperature ranging from 28-32ºC to allow the isolated fungi to adapt.

One week after soil infestation, five surface sterilized seeds were planted in each pot having infested and non-infested soil. Pots were kept in the greenhouse and disease symptoms were monitored daily. Disease symptoms started three weeks after seed sowing and symptoms were similar to that of the plants identified in the field.

The percent disease incidence was recorded 45 days after infestation using formula described by (Nutter *et al*., 1991). The mean disease grading of each isolate in the pathogenicity test were determined on 20 replicated seedlings (5 seedlings per pot/replicate). Individual seedlings were uprooted and fungal potency was estimated for root disease severity following Ziedan (2000) method with a slight modification at a scale of 0-4 where 0 =healthy roots, 1 = < 25% of the root rotting, 2 = 25–50% of the root rotted, 3 = up to 75% of the root rotted, 4 = death of the whole plant. Mean scores of 0: 1-5% NP: considered non-pathogenic, 1: 6-25% LV low virulent, 2: 25-50% MiV: mildly virulent, 3: up to 75% MV: moderately virulent, scores, 4: > 75% HV: highly virulent. Percent disease severity index was calculated by converting the disease severity rating based on above scale values obtained by pathogenicity test by using equation suggested by (Wheeler, 1969).

$$P = \frac{\sum(n.v)}{N.Z} \times 100$$

Where
P = Disease severity %
n = no. of roots for each category
v = Score for attack category
N = No. of roots observed
Z = Score for the highest category

The pathogens were re-isolated from rotted roots and verified by comparing with original culture of isolates and it was found to be similar with respect to all morphological characteristics.

Identification of pathogens: The developed fungal growth was identified up to species level, based on their cultural and morphological characters by compound microscope at (100X) using standard procedures and by identification key described in (a)"Illustrated Genera of Imperfect Fungi" (Barnett & Hunter, 1998), (b) *Fusarium* Laboratory guide to the identification of the major species (Booth, 1977), (c) Compendium of soil fungi (Domsch *et al*., 2007) (d) "A Manual of the Aspergilli" (Thorn & Raper, 1945).

For Macro and microscopic identification, following morphological characteristics were evaluated: fungal colony color, texture and growth of the mycelium, hyphae shape, presence/absence of chlamydospores, shape and size of macro and micro-conidia. Identification of *Sclerotium* spp. was done on the pattern of sclerotia production, shape, color, size and the number of micro-sclerotia according to the keys (Aycock, 1966). The size of sclerotia was measured with a screw gauge, and colors were assigned with the help of Mycological Color Chart (Rayner, 1970). The isolates were grouped according to their identical colony characteristics.

Statistical analysis

The statistical analysis of variance was calculated using statistical 8.1 software. Means and standard errors were compared using methods of Fisher's least significant difference test (LSD) at 5% level of significance. For all pathogenic isolates, the meaning DSI and DI was used to categorize their relative virulence.

Result and Discussion

Survey of root rot incidence: The outcomes of the current study revealed that root-rot disease of peanut was widespread in peanut growing areas of Pothwar region of Punjab province. Overall, root rots were noticed in peanut plants in more than 60% of surveyed fields. A roving survey was conducted during 2019-2020 in the Gujarkhan, Attock, Jand, Pindigheb, Faitha Jang, Chakwal and Talagang Tehsils to assess the incidence and severity of root rot of peanut. Peanut cultivation area in tehsil Attock, FatehJang, Pindi Gheb, Jand, Gujar Khan, Chakwal and Talagang was distributed as, 3,439.9; 2,237.9; 8,656.4; 6,475.1; 5,949; 20,882.2 and 25,212.4 hectares respectively (Anon., 2020b).

The root rot disease was prevalent (61.25%) in the locations surveyed across 7 tehsils (Gujarkhan (46.66%), Attock (83.33 %), Jand (54.54%), PindiGheb (58.33%), Faitha Jang (87.5%), Chakwal (58.33%) and Talagang (50.0%)) of three districts. The mean root rot disease incidence in the 79 surveyed villages ranged from 3.2% to 32.0% in 2019 and from 3.2% to 33.0% in 2020 (Fig. 1).

For incidence and severity of the peanut root rot disease, significant differences (p<0.05) were observed amongst the locations surveyed in both years. For the year 2019, among all the tehsils, maximum disease incidence was detected in Attock (14.2%) followed by Chakwal (13.1%), Jand (12.0%) and Pindi gheb (12.0%) whereas least disease incidence was noticed in Gujarkhan (8.0%). In tehsils Faitha Jang and Talagang disease incidence was (11.7%) and (10.2%) respectively. While for the year 2020, maximum disease incidence was also in tehsil Attock (15.9%) followed by Faitha Jang, Jand, Chakwal and Pindi gheb with 14.9%, 14.3%, 14.1% and 13.8% respectively and least incidence (9.6%) was recorded in tehsil Gujarkhan. During both years, disease incidence in Chakwal, Jand and Pindigheb did not significantly (p<0.05) differ from each other but was significantly differed from other 4 tehsils. Similarly in case of percent disease severity, maximum severity during 2019 was in tehsil Faitha Jang (20.4%) followed by Attock, Pindigheb, Jand and Chakwal and minimum was (10%) in tehsil Gujarkhan. During 2020, maximum disease severity was in tehsil Attock (20.7%) followed by Faitha Jang, Jand, Chakwal and Pindigheb and minimum was (10.1%) in Gujarkhan. Disease severity in both the years was not significantly (p<0.05) in tehsil Chakwal, Jand and Pindigheb but was significantly (p<0.05) different from Attock, Talagang and Gujarkhan (Table 2).

There was no significant difference (p>0.05) in disease incidence between 2019 and 2020 ($t_{0.05}(3) = 1.97$, p<0.05) and disease severity of root rot disease for both years 2019 and 2020 ($f_{0.05} = 3.72$, p<0.05).

In Gujarkhan tehsil, disease incidence was high in village Chak Maradi (22.5%) and (23.2%) and lowest in village Qatbal (2.7%) and Dhok Sakrila (3.5%) during 2019 and 2020. The disease incidence in Chak Maradi was significantly (p<0.05) high than all the other villages including Jatli (17%), mohra (14.9%), Sarahadny (14.2%) and Sukho (12.2%). However, it differed significantly (p<0.05) from Phimbal (3.8%) and Thakramore (2.8%). The maximum disease severity was observed in Chak Maradi (26.1%) and Nata (28.6%) and lowest was in Dhok Sakrila, Phimbal (2.2%) and Thakra more (3.3%) during 2019 and 2020. In Chak Maradi, Jatli and Nata disease severity differed significantly (p<0.05) from all other surveyed villages in Gujarkhan (Fig. 2).

DI; Disease incidence, DS; Disease severity: Different letters for mean values denote statistically the significant difference (LSD, p<0.05).

In Attock, infected fields were observed in 15 villages in 2019 and 2020. The percent disease incidence was highest at China Chok, Kamra Kalan and Kisra villages during both survey years, ranging from 20.5% to 22.3%, while Graa village had the lowest incidence, ranging from 3.9% to 5.4%. The percent disease incidence significantly differed among villages in both years. During the same period, the severity of the disease varied significantly (p<0.05) among villages, with Kisra village experiencing the highest severity at 33.0% and Graa village the lowest at 3.8%. Karma (28.9%), Kamra kalan (26.9%), Peanut Research Station Attock (26.6%), China Chok (23.3%), Mirza (22.9%), Ghazi Brotha (21.5%) and Shakardara (20.8%) exhibited high root rot disease severity, significantly differing (p<0.05) from all other villages. (Fig. 3).

DI; Disease incidence, DS; Disease severity: During 2019 in tehsil Jand, the highest disease incidence was observed at Kot Chaji Jalwal village (32.0%), significantly differing (p<0.05) from all other villages. However, disease incidence did not significantly differ (p<0.05) in villages Chura Sharif, Kotchagi, Sagri, Olive Garden Sagri and village Soghria exhibited the lowest incidence. In 2020, the maximum disease was 36.1% at Kot Chaji Jalwal village, and the minimum was 3.9% at Soghria. Nai Abadi, Saghri and Mariala villages did not significantly differ in disease incidence, but they were significantly different from Chura Sharif, Kotchagi, Soghria and Olive Garden Sagri as in 2019.

Maximum disease severity was at Kot Chaji Jalwal village (39.1%) and (41.1%) and minimum was at olive garden Saghri (3.3%) and at Chura Sharif (5.5%) during 2019 and 2020 respectively. There was no significant (p<0.05) difference in disease severity of villages Chura Sharif, Kotchaji, saghri and Soghria, but there was a significant difference in disease severity of all other villages at 2019. While in 2020, maximum disease severity at Kot Chaji Jalwal (41.1%) was significantly different from all other villages but there was no significant difference in diseases severity at Chura Sharif, Kotchaji, saghri and Soghria villages (Fig. 4).

Table 2. % Disease incidence and severity of root rot disease of peanut across Tehsils in Pothwar Region in 2019 and 2020.

Tehsils	% Disease incidence		% Disease severity	
	2019	2020	2019	2020
Attock	14.2 ± 1.3 A	15.9 ± 1.3 A	18.9 ± 1.7 AB	20.7 ± 1.7 A
Chakwal	13.1 ± 1.2 AB	14.1 ± 1.3 AB	16.5 ± 1.7 AB	18.1 ± 1.7 AB
Faitha Jang	11.7 ± 1.5 ABC	14.9 ± 1.6 AB	20.4 ± 2.1 A	20.3 ± 2.0 AB
Jand	12.0 ± 1.4 AB	14.3 ± 1.5 AB	16.6 ± 1.9 AB	18.4 ± 1.8 AB
Pindi gheb	12.0 ± 1.3 AB	13.8 ± 1.3 AB	16.7 ± 1.7 AB	17.2 ± 1.7 AB
Talagang	10.2 ± 1.4 BC	11.6 ± 1.5 BC	14.7 ± 1.9 BC	15.6 ± 1.8 B
Gujarkhan	8.0 ± 1.2 C	9.6 ± 1.4 C	10 ± 1.6 C	10.1 ± 1.5 C

1- Different letters for mean values denote statistically the significant difference (LSD, p<0.05)
2- Mean triplicates ± standard error. (Value ± SE)

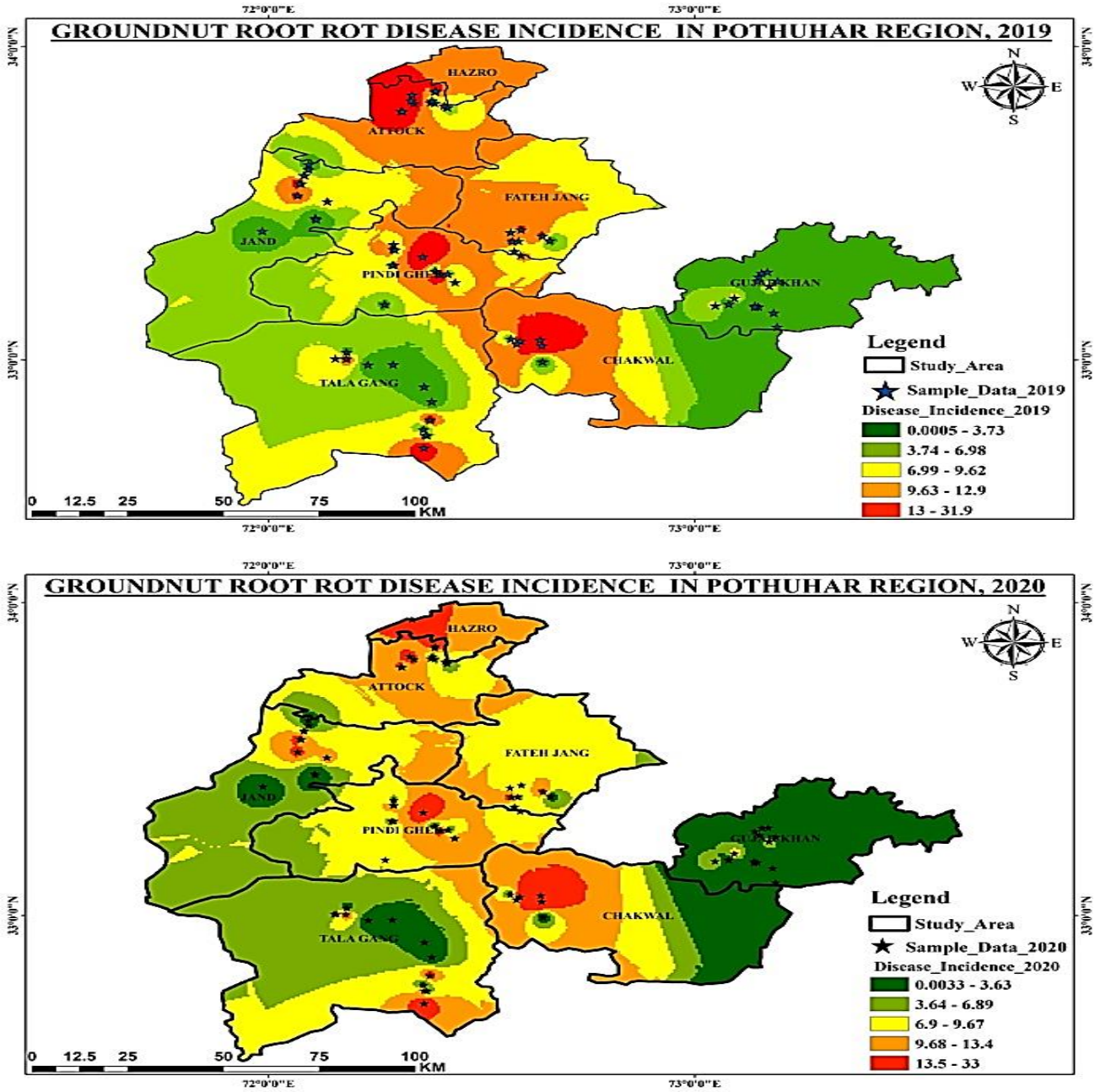

Fig. 1. Map of Pothwar Region of Punjab, Pakistan showing disease incidence (%), different survey and sampling sites during cropping season 2019 & 2020.

DI; Disease incidence, DS; Disease severity: In tehsil Pindigheb during 2019, a disease incidence of 32.0% was noted as the highest and a lowest was 3.1%. Dhok Inayat recorded the high incidence of the root rot disease, and differed significantly (p<0.05) from all other villages. Langrial village showed lowest disease incidence but was not significantly (p<0.05) different from Thathi Karla, Moza Rattarian, Moza Khaur and Makya. In 2020 the root rot disease incidence was very high (32.4%) in Dhok Ianyat and lowest in Langrial (4.3%). The disease incidence in Dhok Inayat was significantly (p<0.05) higher than all other villages including Makial (23.7%) and Thalikarla (21.7%). It however differed significantly (p<0.05) from all other villages. The lowest disease incidence in Langrial was not significantly different from Moza Rattarian and Makyal villages.

In both years, significant differences in disease severity (p<0.05) were recorded among the surveyed villages for root rot disease. Although Thalli Karla (32.7%) recorded the highest severity of the disease but it did not differ significantly (p<0.05) form Dhoke Inayat (30.4%), however, it was significantly different from Makial (28.9%) and Khaur (26.9%). The lowest disease severity was noted in Thali karla (3.1%) which did not significantly differ from Moza Rattarian (3.7%), Moza Khaur (3.7%), Langrial (4.4%) and Makyal (4%) in 2019. In 2020, the disease severity was highest (33.0%) in Thali Karla village but did not differ significantly (p<0.05) from Makial (30.3%), Dhok Inyat (29.7%) and Khaur (29.0%). Lowest disease severity was in Moza Khaur (4.5%) but was not significantly varied from Thali karla (4.7%), Langrial (5%), Makyal (5.1%) and Moza Rattaian (5.6%) (Fig. 5).

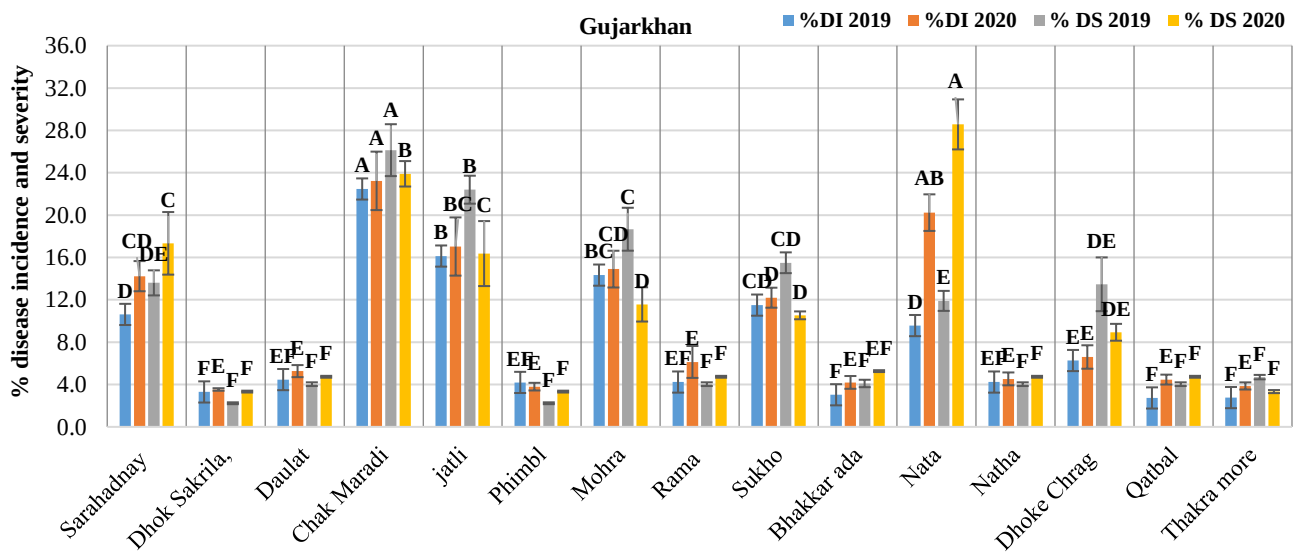

Fig. 2. % Disease incidence and severity of root rot disease of peanut in Tehsil Gujarkhan during 2019 and 2020.

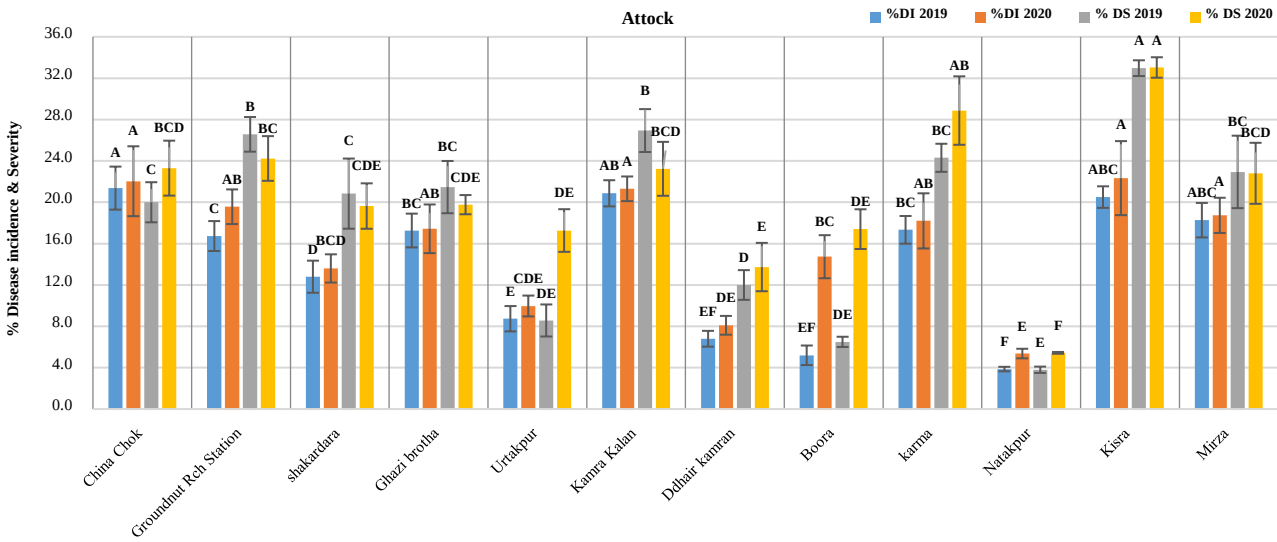

Fig. 3. % Disease incidence and severity of root rot disease of peanut in Tehsil Attock during 2019 and 2020.

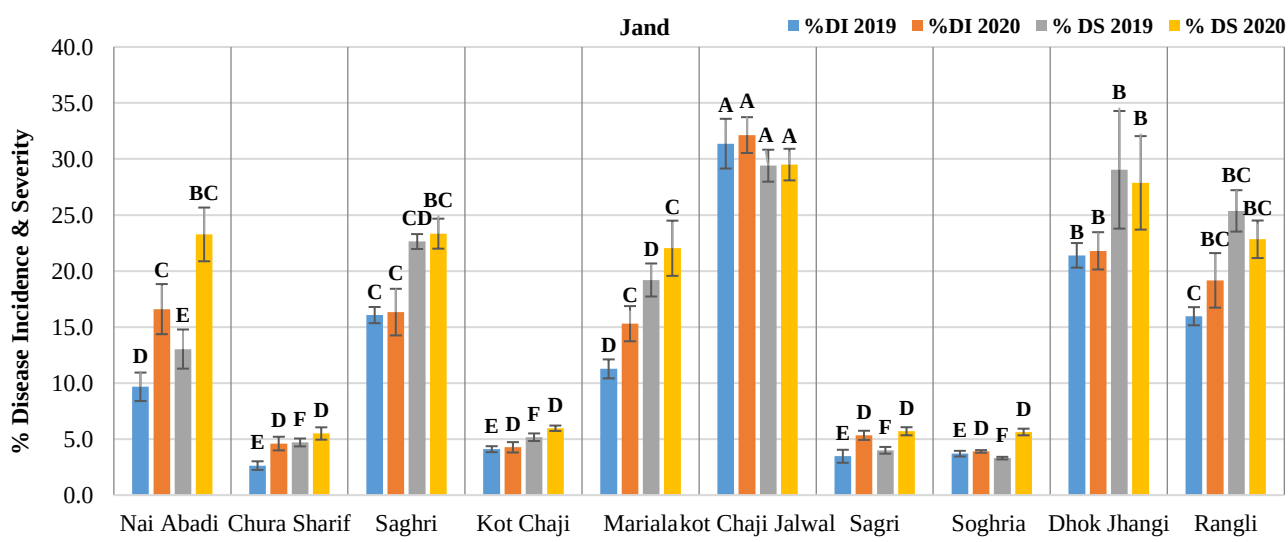

Fig. 4. % Disease incidence and severity of root rot disease of peanut in Tehsil Jand during 2019 and 2020.

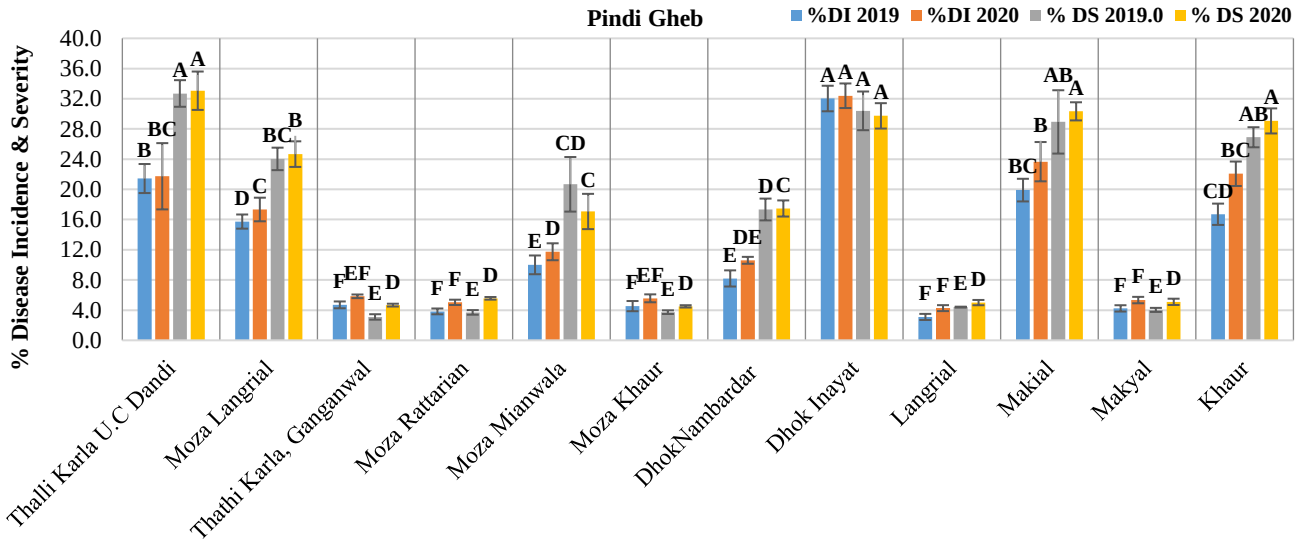

Fig. 5. % Disease incidence and severity of root rot disease of peanut in Tehsil Pindigheb during 2019 and 2020.

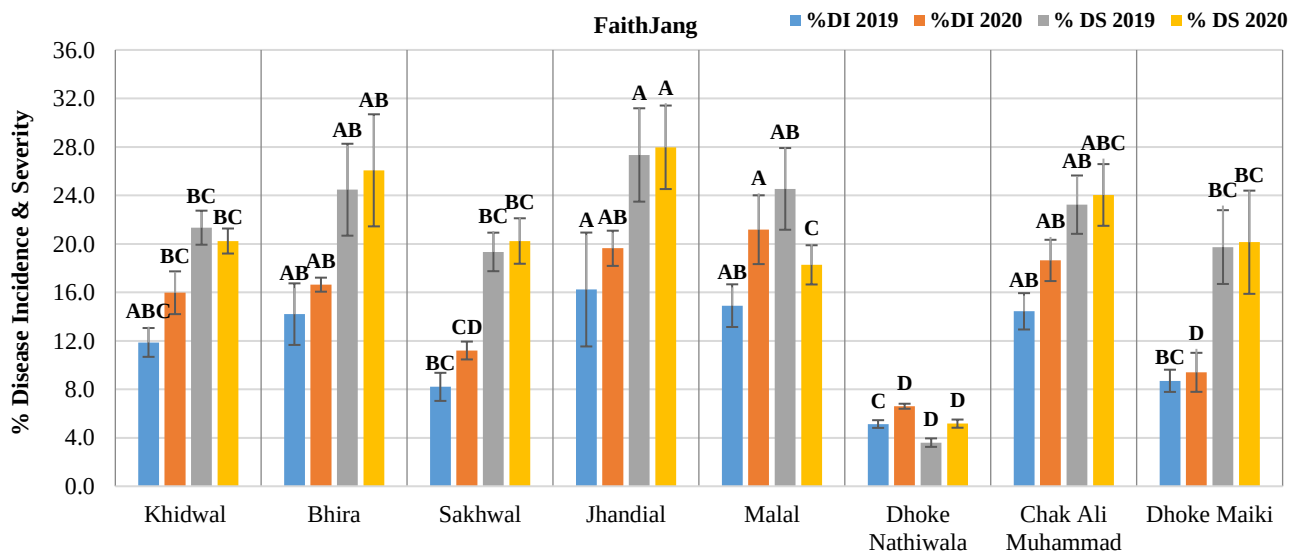

Fig. 6. % Disease incidence and severity of root rot disease of peanut in Tehsil FaithJang during 2019 and 2020.

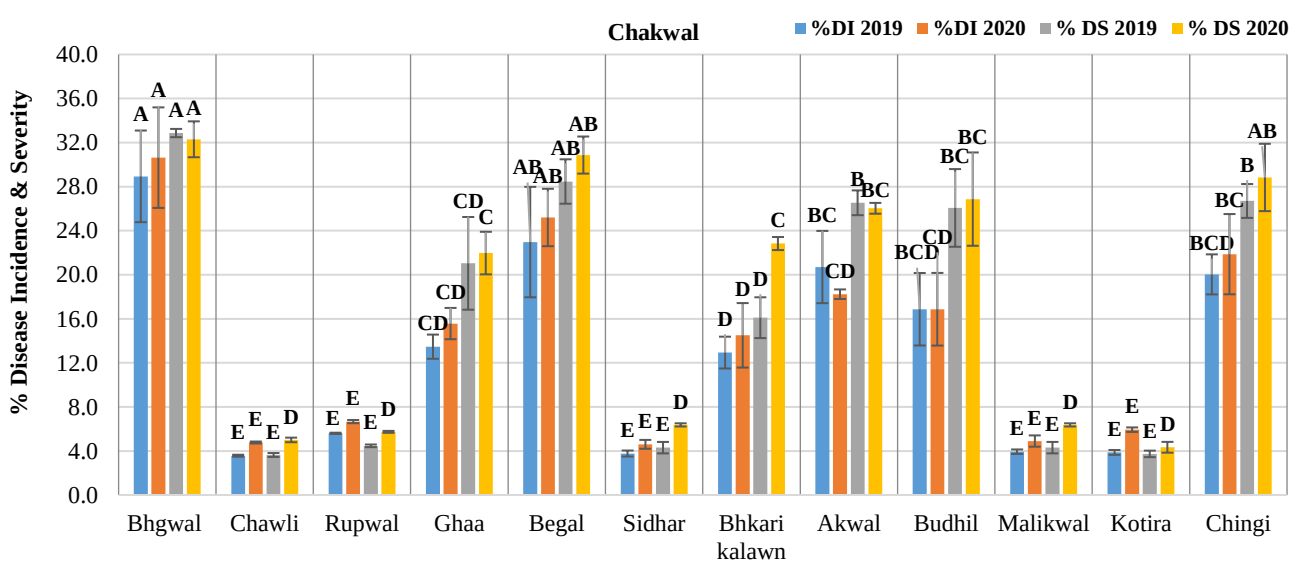

Fig. 7. % Disease incidence and severity of root rot disease of peanut in Tehsil Chakwal during 2019 and 2020.

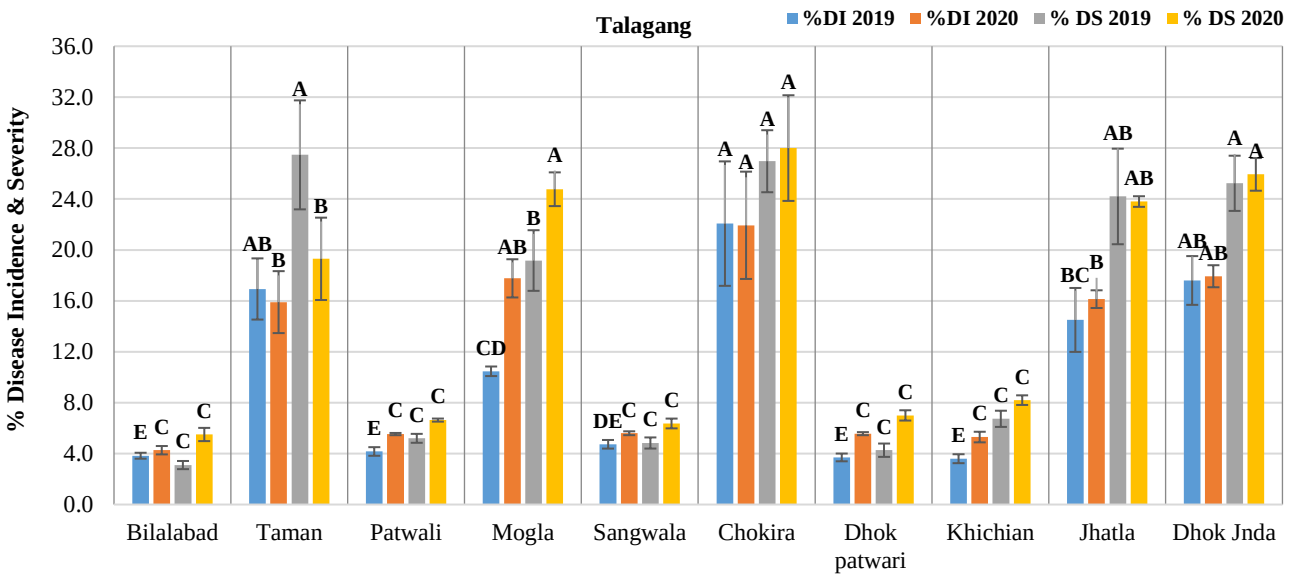

Fig. 8. % Disease incidence and severity of root rot disease of peanut in Tehsil Talagang during 2019 and 2020.

DI; Disease incidence, DS; Disease severity: In FaithJang Tehsil, the highest average root rot disease incidence was noted in Jhandial village (16.2%), while the least average incidence (5.1%) was recorded in Dhoke Nathiwala village. Disease incidence in villages Bhira (14.2%), Malal (14.9%) and Chak Ali (14.4%) did not significantly differ (p<0.05) from each other but differed significantly from all other villages in 2019. In 2020, the highest disease incidence was recorded in Malal (21.2%), while the lowest incidence (6.6%) was in Dhoke Nathiwala village. There was no significant difference in disease incidence among villages Bhira, Jhandial and Chak Ali but it differed from all other villages.

Significant differences (p<0.05) in disease severity were also detected among the villages surveyed for root rot disease in 2019. Jhandial village recorded the maximum severity of the disease (27.3%), while the lowest severity was recorded in Dhok Nathiwala (3.6%). Jhandial significantly differed (p<0.05) form Malal and Bhira (24.5%), Chak Ali Muhammad (23.2%), and Khidwal (21.3%). In 2020, Jhandial village had the maximum disease severity (28.0%), followed by Bhira village (26.1%), Chak Ali Muhammad (24.0%), and Khidwal, Sakhwal, and Dhoke Maiki (20.2%). There was no significant difference in disease severity among Khidwal, Sakhwal, and Dhoke Maiki villages during the same year of the survey (Fig. 6).

DI; Disease incidence, DS; Disease severity: Among the villages surveyed in tehsil Chakwal, percent disease incidence was maximum at Bhagwal village during both the years of survey (28.9% and 30.6%) while, it was minimum with (3.6% and 4.8%) at Chawli village. Disease incidence in Bhagwal village vary significantly (p<0.05) from all other villages surveyed, but in the villages Chawli, Rupwal, Sidhar, Malikwal and Chingi there was no significant difference (p<0.05) in disease incidence during 2019 and 2020. During the same period and under the same environmental conditions, in Chawli village (3.6%) noted the lowest severity. Severity was highest in Bhgwal (32.9%) and varied significantly (p<0.05) from all other villages. Like disease incidence during 2019 and 2020, disease severity was not significantly different in villages Chawli, Rupwal, Sidhar, Malikwal and Chingi that showed the lowest severity (Fig. 7).

DI; Disease incidence, DS; Disease severity: Among all the surveyed villages in tehsil Talagang, the highest disease incidence (22.1%) and (21.9%) of root rot was noted in Chokira village and minimum in Khichian village (3.6%) and Bilalabad village (4.3%) during 2019 and 2020. Disease incidence was highest in Chokira village (22.1%) and was significantly (p<0.05) higher than all other villages including Dhok patwar (3.7%), which recorded the lowest in 2019 and Kitchran (5.3%) in 2020.

The same trend was also noticed with mean disease severity, which ranged from 3.1% in Bilalabad to 27.5% in Taman village during 2019. In 2020, it ranged from 5.5% in Bilalabad to 28.0% in Chokira village. The highest severity was in Chokira village and also was significantly higher from all other villages during both the years. Disease severity in 2020 was greater, than in 2019 cropping season (Fig. 8).

DI; Disease incidence, DS; Disease severity: Disease incidence was more severe in sandy loam as compared to clay or clay loam soils. High disease prevalence in tehsils Attock and FatehJang indicate that root rot pathogens in this area are more aggressive and soil texture, climatic conditions and cultivation of high susceptible cultivar No.334 favor the pathogens to develop more disease.

However comparatively low incidence of root rot in tehsil Gujarkhan was attributable to the cropping systems i.e late sowing (15 May-15 June), cultivation of approved peanut varieties, applying the appropriate concentration of Potash, micronutrients, organic matter in soil and two-time use of gypsum in practice.

Fungal isolation and identification: Isolated 104 fungal isolates initially identified using cultural and morphological characteristic following (Aycock, 1966; Booth, 1977;

Barnett & Hunter, 1998; Domsch *et al*., 2007). These were isolated from 265 samples of damaged roots of peanut plants. The identity of sporulating isolates were verified to three genera; *Fusarium, Athelia* and *Aspergillus* with frequency 29.8%, 49.0% and 21.2% respectively. From total of 104 isolates, 22 were *Aspergillus terreus*, 31 *Fusarium fujikuroi* and 51 as *Athelia rolfsii* according to their identical cultural and morphological characters.

The results of cultural and morphological characteristics study revealed that all isolates of each genus showed similar degrees of growth pattern, shape, pigmentation, colony color and size of the mycelium, shape and size of macro and microconidia and Chlamydospores and shape, size, pattern of sclerotia production and the number of micro-sclerotia.

For *Fusarium fujikuroi* identification all isolates of that fungus showed similar characters and were characterized by a white cottony mycelium that turned pinkish after 5 days when grown on potato dextrose agar (PDA). To reverse dark orange pigment was observed after 7 days of incubation. Macroconidia were relatively slender in shape, normally 3-to-5 septa and medium length with no significant curvature and size of 27-43 ×3.0-4.8µm. Microconidia were oval shaped with 0 to 1 septum, size of 6-11 ×2.0-3.4µm. Sporodochia and Chlamydospores were absent (Leslie & Summerell, 2006).

Athelia rolfsii (anamorph: *Sclerotium rolfsii* Sacc.) showed two types of colonies i.e fluffy and compact, showing white color mycelia. The average colony growth rate was 1.3–1.7 mm/day. Conidia were not formed. Hyphae was pale brown or brown and branched. Side branches were septate, constricted closely near the central hyphae, sometimes with clamp connections in isolates. Mycelium was 3.5–9.6 µm in diameter. There was no variance in the color of sclerotial bodies of all these isolates. Round sclerotia started to grow 8 days after incubation. During the early stage sclerotia color were whitish but it changed to brownish as they matured. Size of Sclerotia varied from 0.4 to 2.2 mm in diameter. Hyphae were (4.5–) 6–9 (–12) µm wide (Aycock, 1966).

Colony color of *Aspergillus terreus* isolates was light yellow to dark orange brown and reverse to light brown. Colony attained 33 to 47mm in diameter after 7 day of incubation at 26°C. Mycelium was wide and septate. Conidial heads shape was as globose to slightly elliptical, 70-160µ long by 40-60µ wide. Conidiophore were long, smooth, hyaline, 100-215µ long and 5-6µ wide. Conidia globose, 1.8 − 2.5µ in diameter. Vesicle globose, 12-19µ in diameter. Matulae and Phialides were ampuliform 5 − 6µ by 1.5 − 2µ wide. On the hyphae some hyaline accessory conidia were also visible. *A. terreus* is the only member of the genus Aspergillus that produces such structures (Balajee, 2009).

Pathogenicity test: Pathogens, isolated from diseased plant roots, were tested for their pathogenicity in peanut seedlings. The result showed that early symptom development began with reddish brown lesion on the top, wilting of the lower leaves and vascular tissues of roots starts to turn brown. Later on the whole plant leaves wilted, roots rotted and the plant died. The control plants were healthy and showed no symptoms.

All fungal isolates used in the experiment were pathogenic to detached peanut roots with various percentage of disease incidence (DI) and severity index (DSI). Pathogenicity of *Athelia rolfsii*, *Fusarium fujikuroi* and *Aspergillus terreus* isolates were evaluated on peanut cultivar No.334 under growth chamber conditions. The disease incidence and severity values of 104 isolates varied between 15.33% to 93.3% and 13.53% to 89.53% respectively. The FGR2, CHGRF5, ATGR3, PGGR6, PGGR1, CHGR4, FGR, TGGR7 and CHGR2a isolates caused disease incidence and severity values were significantly higher than all other isolates (Table 3).

Thirty-one (31) *Fusarium fujikuroi* isolates had a high difference in percent disease incidence that ranged from 24.4% (FGR6) to 88.9% (CHGRF5) and percent disease severity index ranged from 19.7% (FGR6) to 84.2% (FGR). Among, 51 isolates of *Athelia rolfsii*, the isolate CHGR2a recorded the maximum disease incidence and severity of 93.3% and 89.5%, while the isolate PGGR recorded the least disease incidence and severity of 15.53% and 13.53% respectively. Disease incidence values of 22 isolates of *Aspergillus terreus* varied between 24.4% of isolate CHGR5 to 84.4% of isolate CHGR4. Similarly disease severity varied between 20.6% of isolate CHGR5 to 79.7% of isolates CHGR4 in same conditions. The highest disease incidence was recorded by isolate CHGR2a (93.3%), while the highest disease severity was recorded by same isolate was 89.5%.

Based on disease severity, these isolates were grouped into five categories of virulence: Scores of 0: 1-5% NP: considered non-pathogenic, 1: 6-25% LV low virulent, 2: 25-50% MiV: mildly virulent, 3: up to 75% MV: moderately virulent, scores, 4: > 75% HV: highly virulent. Nine (9) isolates were highly virulent, eighteen (18) showed moderate virulence, sixty-nine (69) were mildly virulent and eight (8) isolates were in the 6-25% (LV) range. No isolate was in range of 1-5% i.e., Nonpathogenic (NP).

The ANOVA results of disease incidence and severity indicated highly significant differences ($p<0.05$) among inoculated fungal isolates of all species. The pathogenicity tests revealed that *A. rolfsii* and *F. fujikuroi* isolates proved to be the most and almost equally virulent pathogens ($p<0.05$). Various isolates of both species caused significant damage, with whole plants died in some pots. Most isolates of *A. terreus*, on the other hand, were moderately virulent on root rot, inducing necrosis or rot at lesser levels as *F. fujikuori* and *A. rolfsii* isolates. Hence, most of the isolates significantly affected the percentage of disease severity and incidence (Table 3).

Three isolates of *A. rolfsii* and *F. fujikuroi* and two isolates of *A. terreus* were highly virulent, caused more than 80% disease severity and also caused extensive damage and the whole plants died in some pots. *F. fujikuroi* species 4 isolates were highly virulent, 4 were moderately virulent, 21 were mildly virulent and 2 were less virulent. Similarly in case of *A. rolfsii* species 4 isolates were highly virulent, 10 were moderately virulent, 33 were mildly virulent and 4 isolates were less virulent. Likewise, in case of *A.terreus* 2 isolates were highly virulent, 2 were moderately virulent, 15 were mildly virulent and 3 were less virulent.

To confirm the Koch's Postulate, all the pathogens were re-isolated from infected plant parts, and it was confirmed that the morphological characters of the pathogens were the same as the inoculated isolates. The pathogens were not isolated from un-inoculated control plants.

Table 3. The pathogenicity of the 104 fungal isolates on peanut cultivar No. 334 plants identified in the present study.

Tehsils	Village	Isolate	Pathogen	% Disease incidence	% Disease severity	Isolates	Pathogen	% Disease incidence	% Disease severity
	Sarahadnay	ATGR1	A.r	35.5M-O	29.76Q-U	CHGRF1	F.f	35.53 J-M	30.53O-U
	Maradi	FGR10	F.f	31.1N-P	25.9R-V	TGGR1	A.t	28.83 J-M	24.8STU
	Jatli	CHGR1	A.t	37.8L-N	32.93O-S	CHGR1a	A.r	35.53J-M	32.1M-T
Gujarkhn	Mohra Khir	ATGR2	A.r	24.4P	20.36V	CHGRF2	F.f	31.07LM	26.1Q-U
	Sukho	FGR1	F.f	37.8L-N	31.2P-U	TGGR2	A.t	35.53J-M	31.3M-U
	Nata Mohra	CHGR2	A.t	48.9H-K	44.23J-M	TGGR3	A.t	35.53J-M	30.4O-U
	Dhok chrag	ATGR17	A.r	68.8B-D	63.56C-D	CHGR2a	A.r	93.30A	89.5A
	China chok	ATGR4	A.r	42.2K-M	38.6L-P	TGGR4	A.t	42.20G-J	37.23I-O
	GRS,attock	FGR2	F.f	84.4A	78.43AB	CHGRF3	F.f	28.83M	23U
	Shakardara	CHGR3	A.t	35.5M-O	29.76Q-U	CHGR3a	A.r	35.53J-M	29.43O-U
	Ghazi broth	ATGR5	A.r	37.8L-N	33.3O-R	CHGRF4	F.f	39.97H-K	34.6K-Q
Attock	Urtakpur	FGR3	F.f	42.2K-M	36.86M-Q	CHGR4a	A.r	51.07EF	45.8F-I
	Kamra kala	ATGR6	A.r	48.9H-K	43.56J-M	CHGR5a	A.r	37.77I-L	34.2L-R
	Ddhirkamra	ATGR7	A.r	55.6F-H	50.66C-J	FGR4	F.f	42.20G-J	37.56I-O
	Kamra	CHGR10	A.t	44.4J-L	41.96KLM	TGGR5	A.t	48.83FG	44.77F-J
	Kisra	ATGR8	A.r	71.1BC	65.13CD	CHGR6a	A.r	35.53J-M	30.03O-U
	Mirza	CHGRF5	F.f	88.9A	83.26AB	CHGRF6	F.f	51.07EF	46.17FGH
	Nai Abadi	ATGR9	A.r	35.5M-O	30.9Q-U	CHGR7a	A.r	31.07LM	25.77RSTU
	Saghri	ATGR10	A.r	46.7I-K	41.5KLMN	TGGR6	A.t	68.83C	63.4D
	Mariala	CHGR5	A.t	24.4P	20.6V	CHGR8a	A.r	35.53J-M	29.73O-U
Jand	Kot Chaji	ATGR11	A.r	73.3B	66.5CD	CHGRF7	F.f	42.20G-J	37.5I-O
	Kot Chaji	FGR5	F.f	37.8L-N	32.43P-T	CHGR9a	A.r	33.3K-M	28.53P-U
	Dhok Jhngi	ATGR12	A.r	68.9B-D	63.73CDE	CHGR10a	A.r	57.73DE	52.3EF
	Rangli	ATGR13	A.r	37.8L-N	33.73OPQ	TGGR7	A.t	84.4B	76.3C
	Thalli Krla	ATGR14	A.r	68.9B-D	64.4CD	CHGR11a	A.r	42.2G-J	37.97H-O
	Langrial	CHGR6	A.t	37.8L-N	32.76O-S	CHGRF8	F.f	37.7I-L	32.07M-T
	Mianwala	ATGR15	A.r	35.5M-O	29.9Q-U	PGGR1	A.r	15.5N	13.53V
Pindigheb`	Nambrdar	FGR6	F.f	24.4P	19.7V	PGGR2	A.r	37.7I-L	34.07L-R
	Dhok inayt	ATGR16	A.r	57.8E-G	51.86F-I	TGGR8	A.t	40.0H-K	34.6K-Q
	Dhok inayt	ATGR3	A.r	84.4A	77.4B	PGGR3	A.r	37.7I-L	34.13L-R
	Makial	CHGR7	A.t	37.8L-N	33.26O-R	CHGRF9	F.f	35.5J-M	31.57M-U
	Khaur	ATGR18	A.r	68.9B-D	62.56CDE	PGGR4	A.r	44.4F-I	39.23H-N
	Khidwal	FGR7	F.f	28.9OP	23.8UV	CHGRF10	F.f	28.8M	23.6TU
	Dhok Bhira	ATGR19	F.f	37.8L-N	34.16N-Q	TGGR9	A.t	44.4F-I	39.57H-M
	Sakhwal	FGR8	F.f	62.2D-F	56.5EFG	PGGR5	A.r	64.4CD	58.3DE
Faithajang	Jhandial	ATGR20	A.r	53.3G-I	50.44G-J	ATGR21	A.r	39.9H-K	34.7K-P
	Malal	CHGR8	A.t	51.1G-IJ	46.8H-K	TGGR10	A.t	31.07LM	25.27STU
	AliMuhammad	PGGR6	A.r	91.1A	85.53A	PGGR7	A.r	28.83M	24.2STU
	Dhok Maiki	ATGR22	A.r	31.1N-P	25.93R-V	CHGRF11	F.f	57.7DE	49.73FG
	Bhagwal	FGR9	F.f	75.5B	68.37C	PGGR8	A.r	48.8FG	42.87G-K
	Bhagwal	ATGR23	A.r	35.5M-O	30.3Q-U	TGGR11	A.t	46.63F-H	41.97G-L
	Ghaa	CHGR9	A.t	24.4P	20.07V	PGGR9	A.r	42.13G-IJ	36.4J-P
Chakwal	Begall	CHGRF12	F.f	44.4J-L	40.07K-O	FGR	F.f	88.8AB	84.2ABC
	Bhkari klan	ATGR24	A.r	48.9H-K	45.3I-L	CHGRF13	F.f	51.07EF	46.2FGH
	Akwal	FGR11	F.f	64.4C-E	59.03DEF	PGGR10	A.r	46.63F-H	41.7G-L
	Budhial	ATGR25	A.r	68.9B-D	63.37CDE	CHGR4	A.t	84.40B	79.7BC
	Chingi	FGR12	F.f	37.8L-N	33.33O-R	PGGR11	A.r	37.73I-L	32.6M-S
	Tamman	FGR13	F.f	31.1N-P	25.43S-V	CHGRF14	F.f	31.07LM	25.2STU
	Mogla	ATGR26	A.r	35.5M-O	29.43Q-U	CHGRF15	F.f	48.83FG	43.9F-J
Talagang	Chokira	TGGR12	A.t	57.8E-G	53.13FGH	PGGR12	A.r	91.07AB	85.3AB
	Jhatla	ATGR27	A.r	31.1N-P	24.9TUV	PGGR13	A.r	35.53J-M	30.97N-U
	Dhk Jhenda	ATGR28	A.r	57.8E-G	51.17G-J	CHGRF16	F.f	42.20G-J	37.67H-O

The inoculated treatments within a column with different letters are significantly different (p<0.05) according to Fisher's least significant difference (LSD) test. HV: Highly virulent, MV: Moderately Virulent, MiV: Mildly Virulent, NP: Non-Pathogenic. A.r*: Athelia rolfsii*, F.f: *Fusarium fujikuroi*, A.t: *Aspergillus terreus*

Discussion

A roving survey was carried out in seven Tehsils of three districts *viz.,* Gujarkhan, Attock, Jand, Pindigheb, Faithajang, Chakwal and Talagang during 2019 – 2020. The data on survey revealed that the incidence of root rot of peanut varied from locality to locality. The mean root rot disease incidence in 79 surveyed villages ranged from 3.2% to 32.0% in 2019 and 3.2% to 33.0% in 2020. For incidence of the peanut root rot disease, significant differences (p<0.05) were observed amongst the locations surveyed in both year. For the year 2019, among all the tehsils, maximum disease incidence was noticed in Attock (14.2%) followed by Chakwal (13.1%), Jand (12.0) and Pindi gheb (12.0%). Least disease incidence was noticed in Gujarkhan (8.0%). While for the year 2020, in tehsil Attock maximum disease incidence was (15.9%) followed by Faitha Jang, Jand, Chakwal and Pindi gheb with 14.9%, 14.3%, 14.1% and 13.8% respectively and least incidence was (9.6%) in tehsil Gujarkhan.

It was detected that the disease incidence was more in sandy loam as compared to clay or clay loam soils. The higher incidence of root rot in these tehsils may be due to continuous cropping and cultivation of high susceptible cultivar No.334, because continuous cultivation of any crop over the season and years will build up inoculum level to such an extent as observed by various researchers (Baswaraj, 2005; Praveen, 2009). In tehsil Attock and Fatehjang mostly monocropping of peanut and cultivation of most susceptible cultivar No-334 from a past of few decades on a large area is in practice. Presence of most pathogenic root infecting fungi has resulted higher incidence of root rot that may reach at an alarming level in the coming years. Therefore, in areas where peanut is monocropped and cultivar No.334 is used, comprehensive strategies should to be implemented in managing this soil borne disease. Knowledge on the incidence and prevalence of root rot disease is important to manage the disease.

The variation in root rot incidence in different areas could be explained based on the difference in virulence of the pathogens *F. fujikori, A. rolfsii and A. terreus* prevalent in their respective areas. This variation in disease incidence and severity may be due to microclimatic factors. These results are in conformity with the outcomes of Ghewande *et al*., (2002) where they reported that soil borne pathogens cause plant death resulting in a patchy crop and can reduce yields up to 40%. Chavan *et al*., (2019) reported that root rot disease incidence was more prevalent in sandy soils compared to clay soils and disease incidence was more in Kharif season crop compared to Rabi season. Palaiah *et al*., (2019) reported that the highest peanut root rot disease incidence was in Koppal at 25.25%. Le *et al*., (2012) reported that the southern blight was identified as the most common root rot because *Sclerotium rolfsii* also causes root and stem rots in peanut crop. Zaman & Shakil, (2012) reported that the highest mean root rot disease incidence was 63% and severity was 2.5 % in Attock and the lowest severity was 1.3 in Chakwal.

However comparatively low incidence of root rot in tehsil Gujarkhan is attributable to the cropping systems i.e., late sowing (15 May-15 June), cultivation of approved peanut varieties, applying the appropriate concentration of potash, micronutrients, organic matter in soil and two-time use of gypsum in practice. Nutritional management for the advantage of plant host and disadvantage of plant pathogen is a new method for plant diseases control (Jones *et al*., 1989; Rush *et al*., 2018). Therefore, in areas where peanut monocropping and cultivar No.334 is practiced, comprehensive strategies should to be implemented to manage this soil borne disease. So, ample knowledge on the incidence of root rot disease is important to manage the disease.

From these results it is clear that the isolates of the pathogens of 3 species shows high level of variability in pathogenicity which may be slight to whole death of the plants. It was found that the main causes of root rot of peanut were *F. fujikuroi, A. rolfsii* and *A. terreus*. Disease incidence values of 104 isolates varied from 15.33% to 93.3%. Disease incidence values of FGR2, CHGRF5, ATGR3, PGGR6, PGGR1, CHGR4, FGR, TGGR7, PGGR12 and CHGR2a isolates was significantly higher than all other isolates (Table 3). For incidence, 31 *Fusarium fujikuroi* isolates had a high difference in percent disease incidence that ranged from 24.4% (FGR6) to 88.9% (CHGRF5). Among, 51 isolates of *Athelia rolfsii,* the isolate CHGR2a showed maximum disease incidence of 93.3%, while the isolate PGGR exhibited the least disease incidence of 15.53%. Disease incidence values of 22 isolates of *Aspergillus terreus* varied between 24.4% of isolate CHGR5 to 84.4% of isolate CHGR4.

Similar finding have been also reported by different researchers from other parts of the world (Helal *et al*., 1994; El–Korashy, 1998; Frank *et al*., 1998; Cilliers *et al*., 2003). Atta- Alla *et al*., (2004) reported that several fungi were found to cause the root rot of peanut in the reclaimed land in El-Behera governorate where peanut was intensively cultivated in Egypt and they also explained that *Rhizoctonia solani*, *Aspergillus niger*, *Fusarium* spp., *Sclerotium rolfsii* and *Macrophomina phaseolina* were prevalent over the collected samples and recovered in frequencies of 74%, 69%, 66%, 48%, and 39%, respectively.

Xie *et al*., (2014), *S. rolfsii* isolates showed differences in virulence inoculated on pepper and tomato. Mahato & Biswas, (2017) reported that 10 isolates of *S. rolfsii* showed variation in the virulence on a single variety of tomato. In a related study, Jebaraj *et al*., (2016) showed the differences in virulence of *S. rolfsii* isolates obtained from different peanut growing areas of India. According to the outcomes of Kumar *et al*., (1997), the most susceptible infection stage of peanut to *S. rolfsii* was at 15 days old plant and the least infection is in 105 days old plants.

Charles & Kenneth (1945), reported that *Aspergillus terreus* is typically a soil organism, especially widespread in warm arable soils, grows well at temperatures of 35 to 37°C. According to Dewan & Sivasithamparam, (1988), *A. terreus* is shown to cause root rot diseases in wheat and *Lolium* species. Louis *et al*., (2014), mentioned that *A. terreus* abundantly produced AC (accessory conidia) and multipolar germinating PC (phialidic conidia) to invade potato leaf tissue. Maryam *et al*., (2015), studied that, in micro-organisms, fungal species of the genus *Aspergillus* were soil

borne micro-organisms, and their prevalence in rhizospheric and non-rhizospheric soils has been widely studied.

Sun *et al*., (2023) reported that root rot of peanut (*Arachis hypogaea* L.), caused by *Fusarium* spp., was a distressing disease in most peanut cultivation areas and *Fusarium* root rot of peanut had been detected in Henan province, China. Sun, (1975), reported that *F. fujikuroi* could infect plants through root tip, seeds and stem base, but not through leaf blade or leaf axil. Disease caused by *F. fujikuroi* can lead to great yield loss ranging from 20 to 50% (Ito & Kimura, 1931). It is reported by (Pedrozo *et al*., 2015) that *F. fujikuroi* caused root rot and wilt on soybean, seedling wilt in cotton (Zhu *et al*., 2021), and bakanae disease in rice (Carter *et al*., 2008). Zhao *et al*., (2020) reported that *F. fujikuroi* causes soybean root rot in china. Detarnaltes *et al*., (2021), mentioned that *F. fujikuroi* causes root rot and seedling elongation of Soybean in Indiana. Species such as *F. verticilloides*, *F. proliferatum* and *F. fujikuroi* have been concerned in diseases of agricultural crops including maize, rice, sugarcane and wheat. Mohammadi *et al*., (2016). Zaman & Shakil, (2012) reported that *Fusarium* spp. are more prevalent pathogens in the main peanut growing areas of Punjab. They attack underground parts of plants leading to pre and post emergence.

The above reports are in accordance with the present study. From the findings of the present study, it can be resulted that the root-rot disease of peanut is an important problem in Pothwar Region of Punjab, Pakistan. As it is perceived throughout personal discussion with the local farmers in the survey area, growers mostly pay less emphasis to a disease until it causes a total death of the plants. Hence, it should be recommended that growers should apply control measures as early as possible before higher disease severity rate occurs. Thus the outcomes of this study can serve as an input for farmers to develop best control strategies for fungal root-rot disease in peanut growing area. This is the first report on root rot of peanut caused by *F. fujikuroi*, *A. rolfsii* and *A. terreus* in Pothwar region of Punjab, Pakistan. The findings in the present study will be also beneficial for disease monitoring, quarantine purposes, breeding program and disease management strategies.

Conclusion

Peanut root rot complex was found to be widespread in Pothwar region of Punjab, Pakistan and incidence of the disease varied among 7 tehsils of 3 districts. The highest mean incidence were recorded in the Attoch Tehsil, followed by Chakwal. *A. rolfsii* was the most frequently isolated pathogen from diseased peanut roots, followed by *F. fujikuroi*, and *A. terreus*. During pathogenicity test, these three pathogens also caused the most severe root rot on peanut plants. Furthermore, the three pathogens combined treatment resulted in increased disease severity. Further studies should be conducted in other peanut growing areas of Pakistan to get a clear picture of root rot etiology in Pakistan. Furthermore, proper early disease management strategies, such as crop rotations, adequate fertilization, cultivar resistance, pest control, tillage practices, weed control, seed treatment and biological control methods, should be adopted by farmers in that study area.

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