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ASSESSMENT OF GUAVA WILT DISEASE (GWD) AND VARIETAL SUSCEPTIBILITY IN PUNJAB-PAKISTAN

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Guava cultivation is rapidly rising in the progressive farming community due to its early bearing habit, long harvesting span and meadow orchard culture. However, in the last 7-8 years, despite 25% increase in the area under cultivation, production has not increased accordingly and per hectare yield has decreased up to 13% mainly due to guava decline which is alarming situation. In this scenario, extensive surveys were conducted to estimate the damage caused by guava wilt disease which is badly affecting guava industry and farming community. Infected trees were found at a much higher proportion in cv. Pyriform (Surahi) in Lahore (52.92%) and Faisalabad (65.12%) regions compared with cv. Round (Gola). Most of the elite strains in both Pyriform and Round cultivars across different regions in central and southern Punjab were found infected with wilt disease. In Pyriform, strains viz. Large Surahi, Small Surahi and Sadabahar Surahi while in cv. Round, Large Gola and Sadabahar Gola strains were more susceptible to wilt compared with other strains. Cytological characterization of isolates recovered from infected roots collected during surveys showed frequent involvement of Fusarium species in guava decline. Further characterization and screening of elite guava strains for tolerance against wilt disease is in progress. Such studies will lead towards identification of guava strains tolerant to GWD for future breeding and biotechnology applications.
Keywords: *Psidium guajava*, guava decline, fruit shape, fusarium, anthracnose

INTRODUCTION

Guava, *Psidium guajava* L., is widely grown in tropical and sub-tropical areas of the world and is known as "Apple of Tropics". Its higher nutritive value, abundant availability, low price compared with other fruit crops and year-round production makes it an attractive candidate crop for the progressive farmers (Usman *et al.*, 2013; Karim *et al*., 2013). Guava fruit contains 2.5 times more ascorbic acid content compared with citrus (Jawaheer *et al.*, 2003). Medicinal significance of guava has been reported to be anti-diabetic, helps in boosting human immunity system, reducing risk of cancer and cardiac arrest, lowers stress and a suitable candidate for weight loss (Sanda *et al.*, 2011).

Among Asian countries, leading guava producers include India and Pakistan with annual production of 4.10 and 0.52million tons, respectively. In Pakistan, guava is grown as fourth most important fruit crop after citrus, mango and apple in terms of area and production (FAO, 2016). Guava is extensively grown in Punjab (~ 80%) due to its higher adaptability and tolerance to diverse agroclimatic conditions (Mehmood *et al*., 2013, 2014). Central Punjab is the hub of guava production including Lahore, Sheikhupura, Faisalabad, Sahiwal and Okara districts. Sheikhupura is main guava growing region having about 5000 hectares under guava production (Anonymous, 2011). Over time, area under guava plantation has been increasing (25%) while during last decade production and per hectare yield (-13.65%) has been declining (Table 1) leading to fluctuating exports to different countries including UAE, Afghanistan, Canada, Italy, Norway and Switzerland (Anonymous, 2015). Particularly, during 2011-2012, there was 7% increase in area under cultivation while production declined by 10% indicating massive removal of bearing guava trees and their replacement with new plantations mainly due to guava decline (Table 1). This

Table 1. Overview of declining guava industry in Pakistan.

Parameters	Years								Net Change (%)
	2008	2009	2010	2011	2012	2013	2014	2015	2008-15
Area (000, ha)	49.70	48.70	48.10	49.30	52.80	53.37	52.69	66.38	25.12
Production (000, tons)	422.00	395.00	390.00	420.00	377.00	379.00	375.00	496.00	14.91
Yield (tons/ha)	8.49	8.11	8.11	8.52	7.14	7.10	7.11	7.47	-13.65

Note: During 2011-2012, observe sharp increase in area (7%) with decrease in production (10%) and per hectare yield (16%) indicating massive removal of bearing guava orchards due to guava decline

situation is highly alarming for the guava industry while growers are highly inclined towards meadow orcharding.

Guava industry of Pakistan is facing many pathological problems including guava wilt disease (GWD), anthracnose and soft end rot. Guava wilt disease and decline has emerged as devastating threat to the global guava industry and has been widely reported in Brazil, Mexico, India, Pakistan, South Africa, and Bangladesh (Vos *et al.,* 1998; Misra and Pandey, 2000; Bokhari *et al.,* 2008; Gomes *et al.,* 2012; Hussain *et al.,* 2012). Guava wilt drastically reduced fruit production in many areas of India like West Bengal where plants have been replaced every five years (Misra, 2006). In Pakistan, GWD have completely removed many highly productive orchards rapidly and has reduced per hectare yield despite increase in the area under cultivation (Hussain, 2012; Usman and Fatima, 2013). The severity of GWD has further increased due to malpractices by the farmers done to avoid summer crop due to fruit fly damage. These include removal of leaves accompanied with little or no irrigation to enhance flower and fruit drop during summer season thus leaving the plant trunks exposed to heat stress. This is followed by heavy irrigation and fertilization during fall and winter seasons for bumper winter crop. Farmers are also intercropping with wheat, cotton, vegetables etc. and irrigation schedules of these crops do not match with guava irrigation requirements. Collectively these factors are intensifying the severity of guava decline (Usman *et al.,* 2014). In GWD effected plants, green leaves turn yellow with mild leaf curling from margins at the terminal branches, which turns reddish at later stages and fell concomitantly. Twigs bear flowers which fail to grow and newly emerged leaves and flowers become dry and fell. Fruit of all affected branches remain unripen/underdeveloped or mummified. The complete plant may defoliate and finally die (Fig. 1). Medium day length and mild temperature may help in vigorous transfer of the disease (Misra and Pandey, 2000). Guava wilt disease appeared soil borne and may spread through nursery stock grown in contaminated soil and resulting in disease transmission from diseased to clean orchards (Usman and Shah, 2013). Hence spread by putative fungal and other soil borne pathogens in guava can be

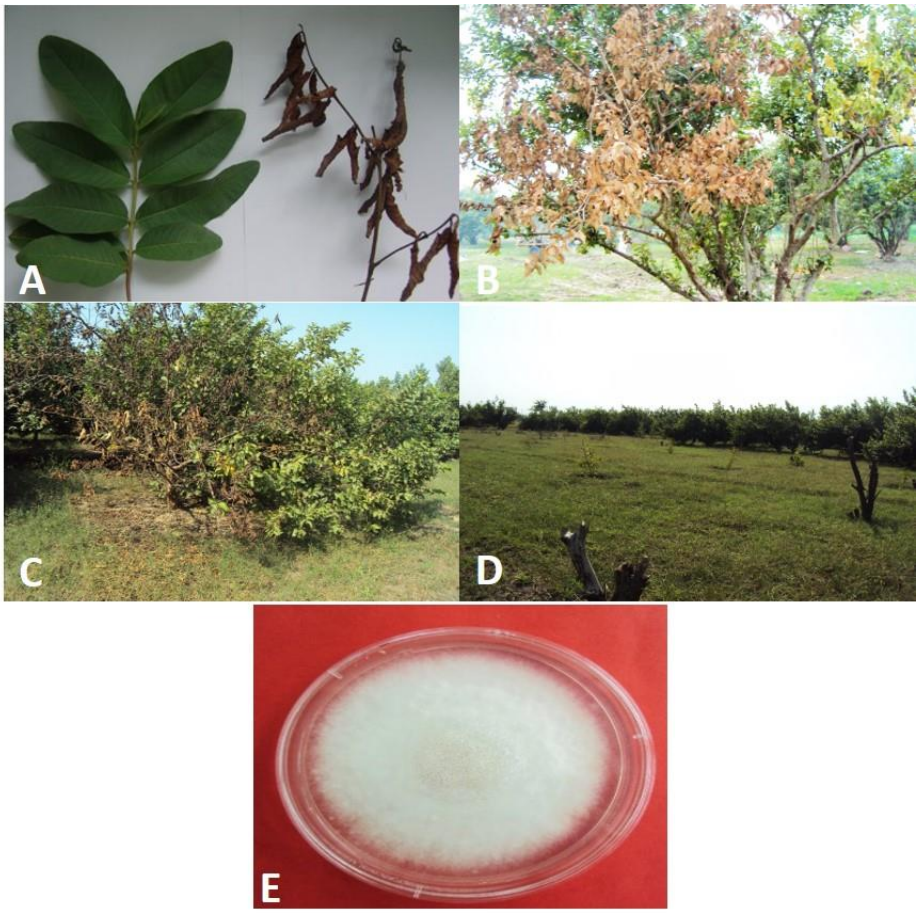

Figure 1. Guava wilt disease in cultivar Pyriform (Surahi) in Sheikhupura-Punjab. Figures show A) healthy and wilted shoot; B-C) wilting mature trees D); commercial orchard after removal of wilted plants and replaced by new plantation without any pesticide treatment and E) *in vitro* culture of *Fusarium* sp. collected from roots of wilting guava plants.

minimized by adopting proper phytosanitary measures in nurseries including use of sterilized potting media and ensure plant sanitation (Usman *et al.,* 2014).

Guava decline has been reported in Pakistan (Ansar *et al.,* 1994; Bokhari *et al.,* 2008); however, these studies were based on extensive surveys. In different regions of Punjab, a high number of orchards have been suffering from this syndrome. Further, no information is available about sensitivity of different guava strains viz. Round (Gola) and Pyriform (Surahi) cultivars to GWD. Above discussion highlights the existing research gaps and need for an extensive study on GWD in potential guava growing regions of Punjab to address this key issue. In Pakistan, different strains in guava cultivars including Round (Gola) and Pyriform (Surahi) are being cultivated for their good taste and size. These strains have been selected by the amateur growers, are seed propagated and show heterozygosity. Little work regarding isolation of putative causal organism of GWD across Punjab province and its cytological and genetic characterization in Pakistan has been reported. Hence, this study was aimed at extensive quantification of disease status through surveying hot spots and assess susceptibility in different cultivars grown in different regions.

MATERIAL AND METHODS

***Plant sampling and disease rating*:** An extensive survey was conducted and information about prevalence of GWD and susceptibility of different guava strains in Round and Pyriform cultivars was collected. Random samples were collected from primary feeding roots in Rhizosphere of the healthy and symptomatic (wilting) guava plants of different strains from following localities across different regions of central and southern Punjab (Table 2). The root samples were preserved immediately in paper bags and labeled for further studies.

Disease intensity was quantified as 25, 50, 75 and 100% infected plants showing wilting using modified rating scale (Cooke, 1998; Madden *et al.,* 2007). Random sampling techniques were used for observing disease symptoms (Hughes and Gottwald, 1998).

Disease incidence was calculated using the following formula

$$\% \text{ Disease incidence} = \frac{\text{No. of infected guava trees}}{\text{Total trees observed}} \text{ x } 100$$

Disease rating scale set as 0; healthy plants, 1; yellowing of the leaves, 2: 25% wilting, 3: 50% wilting, 4: 75% wilting and 5: 100% wilting/completely dead plants was developed for estimating disease severity in Guava (Misra and Pandey, 2000). This disease rating scale was used for random sampling for disease severity estimation in different geographical localities (BWP, MTN, SWL, FSD and LHR) with slight modifications as 1: 25% wilting, 2: 50% wilting, 3: 75% wilting and 4: 100% wilting in plants.

***Data analysis*:** The data was analyzed using Chi square test and Least Significance Differences (LSD) were calculated for Inference after ANOVA (Steel and Torrie, 2004).

RESULTS

***Guava wilt disease incidence in Round and Pyriform cultivars across different regions*:** Guava wilt disease intensity was markedly different (P>0.05) across cultivars and regions in the bearing plants. In healthy plants, higher genotypic differences were observed in Lahore (LHR), Bahawalpur (BWP) and Faisalabad (FSD) regions compared with Multan (MTN) and Sahiwal (SWL) regions. Cultivar Round had higher percentage of healthy plants in LHR (73.94%) and BWP (86.98%) regions compared with cv. Pyriform (47.08%, 66.21%, respectively). Overall, healthy plants were higher in cv. Round (70.32%) compared with cv. Pyriform (52.79%) as presented in Table 3. More wilted plants of cv. Pyriform in LHR (52.92%) and FSD (65.12%) regions suggest that this cv. is more susceptible to GWD compared with cv. Round. Conclusively, cv. Pyriform plants had higher rate of infection by GWD than that of cv. Round plants.

***Guava wilt disease incidence in different regions*:** Intensity of GWD was highly variable (P>0.05) at all levels of infection (%) across different regions. Plant wilting (%) was directly proportional to GWD intensity across different regions in Punjab. Among regions, in FSD region more wilting plants (8%-26%) with higher percentage of wilted plants with higher disease intensities were observed while in LHR region wilt incidence was low and only 7% plants were wilting at lower GWD intensities (Table 4). Trees with 100% infection (wilted plants) were higher (26%) in FSD region followed by LHR region with 15% infected plants. Overall, disease intensity was higher in FSD region (52.04%) compared with LHR (39.08%) and other regions (Table 4).

Table 2. Different sites of guava root sample collections.

Regions	Locations	Localities for sample collection
Lahore (LHR)	31.5546° N 74.3572° E	Sheikhupura, Sharaqpur, Nankana Sahib
Faisalabad (FSD)	31.4187° N 73.0791° E	University of Agriculture, Faisalabad gardens, Samundari, Toba Tek Singh, Gojra, Shahkot
Sahiwal (SWL)	30.6612° N 73.1086° E	Arifwala, Harappa, Chichawatni, Kassowal
Multan (MTN)	30.1984° N 71.4687° E	Lodhran, Khanewal
Bahawalpur (BWP)	29.3957° N 71.6833° E	Chishtian, Hasilpur, Yazman, Ahmadpur East

Table 3. Infected trees (%) showing Guava Wilt Disease in Round and Pyriform cultivars in different regions of Punjab.

Disease rating (%)	Cultivars	BWP	MTN	SWL	FSD	LHR	Overall
Healthy plants	Round	86.98±0.15*	81.74±4.24 NS	72.81±16.51	58.77±6.32*	73.94±3.97**	70.32±3.48**
	Pyriform	66.21±7.76	73.970±4.62	77.92±13.62 NS	34.88±6.57	47.08±4.55	52.79±3.85
Wilting plants	Round	13.02±3.15	18.27±4.23	27.19±16.51 NS	41.23±6.32	26.06±3.97	29.68±3.48
	Pyriform	33.79±7.76*	26.03±4.62 NS	22.08±13.62	65.12±6.57*	52.92±4.55**	47.21±3.85**
25% canopy wilting	Round	3.21±0.69	5.03±2.48 NS	1.88±1.64 NS	4.83±1.18	5.45±1.28	4.43±0.64
	Pyriform	6.22±1.17*	4.25±1.67	0.18±0.18	8.88±1.75*	10.08±1.34*	7.21±0.84**
50% canopy wilting	Round	2.38±0.62	3.78±1.73	3.44±3.31 NS	3.74±1.03	6.84±1.52	4.38±0.72
	Pyriform	8.08±2.07*	5.41±5.84 NS	0.65±0.48	8.93±1.66*	8.84±1.38NS	7.34±0.85**
75% canopy wilting	Round	3.71±1.26	5.67±2.60	2.61±1.68 NS	6.69±1.63	3.63±1.06	4.82±0.79
	Pyriform	7.68±2.09*	5.58±5.46 NS	0.74±0.51	10.48±2.12*	12.31±1.63**	8.80±1.03**
100% canopy wilting	Round	3.72±1.14	3.80±1.84	2.61±1.68	21.68±5.27	10.15±2.15	12.61±2.40
	Pyriform	11.80±3.87*	10.79±2.32 NS	6.22±3.89 NS	31.57±6.21*	21.69±2.99**	20.36±2.63*

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

Table 4. Guava Wilt Disease affected trees (%) in different regions of Punjab.

Regions	Plants canopy wilting (%)				
	25%	50%	75%	100%	Total
Bahawalpur	4.55 ± 0.72^{ab}	4.91 ± 1.17^{ab}	5.47 ± 1.22^{ab}	7.31 ± 2.07^{b}	22.25 ± 4.49^{C}
Multan	4.42 ± 1.34^{ab}	5.05 ± 1.72^{ab}	5.60 ± 1.61^{ab}	9.23 ± 2.06^{b}	24.30 ± 3.77^{BC}
Sahiwal	0.96 ± 0.76^{b}	1.94 ± 1.53^{b}	1.60 ± 0.83^{b}	4.55 ± 2.21^{b}	24.44 ± 10.1^{BC}
Faisalabad	6.66 ± 1.05^{a}	6.09 ± 1.01^{a}	8.40 ± 1.33^{a}	26.15 ± 4.05^{a}	52.04 ± 4.87^{A}
Lahore	7.70 ± 1.00^{a}	7.81 ± 1.03^{a}	7.84 ± 1.22^{a}	15.74 ± 2.07^{b}	39.08 ± 3.79^{B}

Means sharing similar letter in a row or a column are statistically non-significant (P>0.05)

***Guava wilt disease incidence in elite strains of different cultivars*:** A highly genotype dependent response was observed for disease intensity across different strains of Guava cultivars Round and Pyriform. In cultivar Round, plants with 25-50% and 75-100% canopy showing disease intensity were more in strains like Large Gola (39 and 40%) and Sadabahar Gola (18 and 30%) while Green Gola, Chinese Gola and Lucknow White were less susceptible to GWD (Table 5). Similarly, in cultivar Pyriform strains, Sadabahar (25 and 35%) and Large Surahi (17 and 28%) showed higher percentage of diseased plants compared with other strains.

***Guava wilt disease incidence in strains of elite cultivars across different regions*:** Guava wilt disease intensity was different among regions, cultivars and strains. In cv. Pyriform, trees of strains Large Surahi, Sadabahar Surahi and Small Surahi had higher disease intensity across regions indicating more susceptibility of these strains. Trees of Small Surahi were less infected in SWL region. In cv. Round, trees of Large Gola showed higher disease intensity (30 and 20%) in SWL region while no such trend was observed in other regions. These results indicate that both, regions and genotypes are important components showing different GWD intensity responses except few guava strains which had consistently higher disease susceptibility across studied locations (Table 6).

Table 5. Infected trees (%) showing Guava Wilt Disease in strains of elite Guava cultivars in Punjab.

Cultivars	Strains	Plant canopy wilting (%)	
		25-50%	75-100%
Round	Large Gola	38.60 ± 6.04^{a}	40.89 ± 5.80^{a}
	Gola	9.37 ± 0.80^{cd}	15.50 ± 1.63^{c}
	Sadabahar	18.93 ± 0.96^{b}	30.90 ± 3.33^{b}
	Pink Flesh	7.22 ± 1.72^{de}	17.28 ± 0.64^{c}
	Ramzani	13.33 ± 0.75^{bc}	16.67 ± 1.33^{c}
	Lucknow White	3.36 ± 0.19^{de}	3.51 ± 0.46^{de}
	Larkana Red	3.75 ± 0.52^{de}	8.75 ± 0.81^{d}
	Green Gola	2.00 ± 0.35^{e}	0.20 ± 0.01^{e}
	Small Gola	17.71 ± 0.19^{b}	18.94 ± 0.46^{c}
	Chinese Gola	2.50 ± 0.52^{e}	0.00 ± 0.81^{e}
Pyriform	Large Surahi	17.81 ± 3.17^{b}	28.47 ± 2.54^{ab}
	Surahi	13.99 ± 1.42^{b}	25.42 ± 3.12^{b}
	Sadabahar	25.29 ± 1.36^{a}	35.44 ± 2.09^{a}
	Lucknow White	11.46 ± 0.54^{b}	11.24 ± 0.77^{c}
	Small Surahi	13.78 ± 2.48^{b}	19.61 ± 4.49^{bc}
	Larkana Surahi	11.66 ± 1.07^{c}	12.38 ± 0.72^{b}

Means sharing similar letter in a row or a column are statistically non-significant (P>0.05)

***Frequency of fungal growth and fungal species across regions*:** Frequency of fungal isolation and *in vitro* growth was higher in root samples collected from FSD and LHR regions compared with other regions. Among fungi isolates,

Table 6. Infected trees (%) showing Guava Wilt Disease in strains of elite Guava cultivars across different regions of Punjab.

Regions	Varieties	Strains	Plant canopy wilting (%)	
			25-50%	75-100%
Bahawalpur	Round	Gola	8.33±1.20^{c}	15.00±2.98^{b}
		Large Gola	5.50±0.12^{cd}	6.66±1.31^{cd}
		Lucknow White	3.97±0.52^{d}	3.20±0.85^{d}
	Pyriform	Large Surahi	15.19±1.54^{a}	21.85±2.84^{a}
		Larkana Surahi	11.66±1.07^{b}	12.38±0.72^{bc}
Multan	Round	Sadabahar	5.87±1.71^{b}	11.64±1.04^{b}
	Pyriform	Large Surahi	5.00±2.41^{b}	19.00±1.21^{a}
		Small Surahi	14.41±1.91^{a}	15.14±1.28^{ab}
Sahiwal	Round	Large Gola	30.00±2.08^{a}	20.00±1.56^{a}
		Gola	0.33±0.01^{b}	2.81±0.17^{c}
	Pyriform	Large Surahi	3.33±0.29^{b}	20.00±1.39^{a}
		Surrahi	3.14±0.69^{b}	12.50±1.79^{b}
		Small Surahi	0.63±0.07^{b}	0.94±0.12^{c}
Faisalabad	Round	Gola	7.46±0.67^{de}	37.69±2.29^{b}
		Large Gola	6.23±0.57^{e}	10.23±0.64^{d}
		Sadabahar	13.00±2.39^{cd}	23.00±4.31^{c}
		Pink Flesh	11.67±3.43^{cde}	15.83±4.14^{cd}
	Pyriform	Surahi	14.71±1.11^{bc}	47.47±2.65^{a}
		Small Surahi	20.00±0.65^{b}	45.00±2.41^{ab}
		Sadabahar	30.00±2.65^{a}	43.33±2.53^{ab}
Lahore	Round	Large Gola	2.38±1.14^{d}	18.88±0.54^{cd}
		Gola	11.96±2.36^{c}	9.38±1.62^{d}
		Small Gola	17.71±1.31^{ab}	18.94±2.48^{c}
		Sadabahar	21.79±2.47^{a}	24.36±4.32^{bc}
	Pyriform	Large Surahi	15.21±1.71^{bc}	29.88±4.08^{ab}
		Surahi	20.18±0.92^{ab}	35.50±0.86^{a}
		Sadabahar	20.06±1.79^{ab}	35.00±4.91^{a}

Means sharing similar letter in a row or a column are statistically non-significant (P>0.05)

Fusarium species (Fig. 1E) were more abundantly found across different regions especially FSD and LHR regions compared with other fungi (data not shown).

DISCUSSION

Guava wilt disease has been reported throughout guava growing countries including India, China, South Africa, Australia, Brazil and Mexico (Gomes *et al.,* 2012; Hamiduzzaman *et al.,* 1997; Vos *et al.,* 1998) and several causal organisms have been reported. Guava wilt is a complex disease presumably caused by nematode and fungi and was reported in Pakistan by Ansar *et al.,* (1994). However, no extensive survey was conducted to estimate losses inflicted by GWD. To date, no authentic information for identification and disease severity of GWD was available in Pakistan. Hence a precise and comprehensive survey was conducted in the major guava producing regions to assess damage, distribution pattern and varietal susceptibility estimation. Surveying and sampling for disease estimation in fruit crops is an efficient method to get primary information of disease prevalence in a certain area and supports in developing programs for disease management (Narender and Sharma, 2014).

Several surveying methods could be used for estimating disease severity and incidence (Caprioli and Tarantino, 2006). Method of sampling individual trees was developed by *The Central California Tristeza Eradication Agency* and has been used in orchards (Hughes and Gottwald, 1998). Systematic sampling scheme may also be followed for individual trees due to its unbiased estimate of mean population, however, some randomized elements shall also be included. In Pakistan, random sampling can support workers as different varieties of guava are usually planted randomly with no clear varietal distinction. Second method of CCTEA is group sampling of trees called as Group sampling method (Hepworth, 1996). Modified Misra and Pandey (2000) disease rating scale was used in the current study for disease severity estimation effectively. Similar surveys were also conducted in Guava and 176 representative samples were collected from seven different geographical localities (Gupta *et al.,* 2012) for disease estimation.

Safdar *et al.* (2015) conducted preliminary survey for estimating GWD disease prevalence only in district Sheikhupura and Sharaqpur was reported as the most infected area (100% disease prevalence). However, in the current survey, Sheikhupura was a part of LHR region where overall the highest disease prevalence (52%) was noted. These differences in disease severity estimation could be due to more extensive nature of survey in this study. Further, the highest disease incidence found in this region could be due to continuous cultivation of guava in this area for the last several decades with little or no crop rotation. Overall, GWD incidence has been much higher in guava growing regions of Punjab than previously reported. Disease incidence in LHR region was 39% in cv. Round (Gola) which was less than that in FSD (52.04%) and other regions. It was observed in a previous study that cv. Round was more infected compared with cv. Pyriform (Bokahri *et al.*, 2008), however, we found cv. Pyriform and its different strains (Sadabahar Surahi, Small Surahi and Large Surahi) more susceptible to GWD compared with cv. Round and its strains (Sadabahar Gola and Large Gola). The disagreement in findings may be attributed to large scale of the current survey involving different guava strains, disease spread over the years in different areas or due to variation in the disease susceptibility over time.

***Conclusion*:** Guava wilt disease has emerged as a potential threat to guava industry of Pakistan. Such a higher incidence of GWD at all various locations confirms the prevalence of disease. Guava wilt disease incidence was higher in cv. Pyriform (Surahi) compared with cv. Round (Gola). Orchards in LHR and FSD had higher incidence of GWD compared with those in other regions. Elite strains in both cultivars showed higher susceptibility to GWD. Diverse guava germplasm shall be screened to find tolerance against GWD. Frequent isolation of the *Fusarium* species indicates that these species are more likely to be the main cause of GWD in Punjab region. Extensive fungigation program could control Fusarium species and other soil borne pathogens. There is dire need to adapt containerized nursery production system using sterilized soil media to check disease spread from guava nurseries. Further studies on identification and characterization of GWD causing fungal strains is underway.

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