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Short Communication

Involvement of Citrus Nematode (*Tylenchulus semipenetrans* Cobb.) and *Colletotrichum gloeosporioides* (Penz.) in Dieback Affected Orchards of Pakistan

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MUA designed the study, conducted the surveys, executed experimental work and prepared the manuscript. SAK supervised the work. NJ provided technical assistance. AUM analyzed the data.

Key words
Citrus dieback, *Tylenchulus semipenitrans*, *Colletotrichum gloeosporioides*, Punjab

ABSTRACT

Citrus is one of the key fruit produced in Pakistan but facing a major threat of dieback disease. A survey of major citrus growing areas of Punjab province including Sargodha, Silanwali, Kot Momin, Bhalwal, Layyah, T.T Singh and Faisalabad was conducted to assess the disease severity. Five orchards were selected at random from each location for soil, root and twig samples to isolate, purify and identify fungi and nematode. Disease prevalence in Sargodha, Bhalwal, Kot Momin, Silawali and T.T. Singh was 85.98%, 84.64%, 82.88%, 81.79% and 79.23%, respectively which shows that most orchards were infected. Disease incidence was maximum at Kot Momin (5.02 %) followed by Bhalwal (4.05 %) T.T. Singh (3.34 %) and Sargodha (3.35 %), respectively while it was low at Silanwali, Faisalabad and Layyah i.e. 2.31 %, 2.13 % and 1.58 %, respectively. Percent disease index (PDI) was maximum at Bhalwal, T.T. Singh, Sargodha and Kot Momin (35.18%, 32.1 %, 31.81 % and 31.19 %) respectively while was minimum at Silanwali, Layyah, and Faisalabad (29.03 %, 27.26 %, and 25.12 %) respectively. Colonization percentage of the *Colletotrichum gloeosporioides* (Penz.) was found to be highest (42.68 %) in collected twig samples followed by *Colletotrichum acutatum* (14.04 %), *Lasiodiplodia theobromae* (8.42%) and *Phomopsis citri* Fawc (6.87 %). Total number of Nematode/300 ml soil recorded soil recorded from visited locations were maximum in Bhalwal (3814.8) followed by Sargodha (3742.4). Citrus nematodes/300 ml soil and number of J2/5 g of roots were also recorded maximum in Bhalwal (3643.0, 14800) respectively followed by Kot Momin and Sargodha while minimum in Faisalabad. During Survey it was indicated that citrus Dieback is a complex disease caused by the mutual involvement of fungal pathogens and citrus nematode (*Tylenchulus semipenetrans* Cobb.).

Pakistan has 206,569 ha of citrus (*Citrus sinesis* L.) growing area among which Punjab contributed 183210 hectares of area which gives the highest production 2,315,895 tons over all the other provinces because of its favorable environment and growing conditions (Matesz, 2010). Pakistan has potential to produce 18-20 tons per hectare, but the actual yield is 12.78 tons per hectare which is much less than potential (Anon, 2006). This huge gap in yield is due to several cultural and environmental factors as well as infectious diseases, caused by different pathogens. Due to a complex of fungi and nematode plants wilt rapidly. Roots give dirty appearance in presence of soil particles. Roots of citrus crop become rotten and more dirty than healthy roots. Number of insects, pests and diseases attacks on citrus and severely affects the quality and quantity of fruit. Almost 150 pathogens including fungal, bacterial and viral pathogens result in huge yield losses. It is prone to several diseases and dieback is one of the severe and most lethal citrus diseases throughout the world (Alam, 2003; Yesmin *et al*., 2017). Percentage of citrus dieback on Kagzi lime and Elachi lemon was round about 100 % and 89.9 %, respectively (Miah and Fakir, 1987). Dieback is crop limiting factor and become emerging issue of citrus crop in most countries of world. (Rawal and Saxana, 1997).

Citrus nematode (*Tylenchulus semipenetrans* Cobb.) infected citrus orchards in Egypt are known as serious threat for citrus trees. Major symptoms of citrus nematodes are, infected root with less absorption capacity, dieback, less vegetative growth, yellowing of leaves and reduced yield (Bakr *et al*., 2011). Citrus nematode has been observed in citrus with dieback symptoms like yellowing, wilting,

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defoliation and death of twigs and branches. Randhawa (l970) has reported that nutrient imbalance in citrus plants could lead to adverse consequences and ultimately cause the dieback disease of citrus.

Recently, *Colletotrichum gloeosporioides* (Penz.) has been implicated with an outbreak of shoot and twig dieback disease of citrus in the Central Valley of California. Symptoms included leaf chlorosis, crown thinning, gumming on twigs and shoot dieback, and in severe cases, branch dieback of Isolations from symptomatic plant samples frequently yielded *Colletotrichum* species (Joey *et al*., 2017)

Dieback disease is a major threat to citrus industry in Pakistan. There are multiple factors responsible for this disease like fungal pathogens, citrus nematode and nutrients deficiency. Very little information is available on incidence and prevalence of this devastating disease in Pakistan. The present study aims at assesing the citrus dieback incidence, severity and prevalence in different areas of Punjab including Sargodha, T.T. Singh, Layyah and Faisalabad districts.

Material and methods

A total of 35 orchards were surveyed in major citrus producing areas of Sargodha, Silanwali, Kot Momin, Bhalwal, Layyah, T.T Singh and Faisalabad to assess disease prevalence, disease incidence and percent disease index (PDI) (Abedin and Chowdhury, 1982) in 2016-17.

Dieback severity was recorded on a 0-5 scale as described by Rahman and Hossain (1988) with slight modifications, where 0, no infection; 1, up to 10 %; 2, 11-20 %; 3, 21-30 %; 4, 31-50 % and 5, more than 50 % twigs are infected. To confirm the association of pathogens with dyeback and healthy twigs of eight 10-12 years old trees were sampled from each orchard. The samples were brought to laboratory after proper labeling.

The collected samples were used for the isolation of fungi by usual isolation procedure (Ricker and Ricker, 1936). The isolated fungi were purified according to Ellis (1971). The frequency of each fungus isolated from each part from the cultured pieces was calculated using formula of Marasas *et al*. (1988).

Hyphal tips growing out from plated material were transferred to PDA slants and incubated again for 7-8 days at 27°C±2°C. For identification of the isolates from diseased pieces of citrus trees, temporary mounts were prepared, first in distilled water and then in cotton blue lactophenol.

For isolation of nematodes feeder roots along with soil samples were taken from 4-6 inches depth under canopy of each tree. Composite soil sample of 500 ml was collected by mixing 4-5 sub samples in the polythene bag,

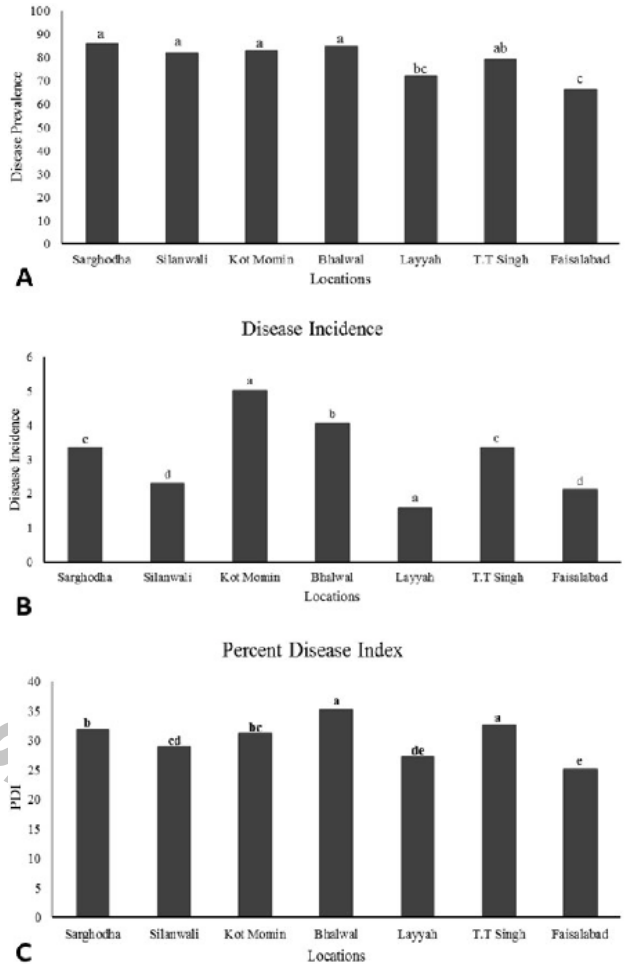

Fig. 1. Percent disease index fungal prevalence (A), incidence (B) and PDI at different locations.

which were sealed loosely to maintain humidity and aeration and stored at 10°C for further studies. Nematodes were isolated from root and soil samples by using Baermann funnel method (McKenry and Roberts, 1985) and Whitehead and Hemming tray method (Whitehead and Hemming, 1965), respectively. Total number of nematode/300 ml soil, citrus nematodes/300 ml soil, stylet bearing/300 ml soil, *Paratylenchus*/300 ml soil, non-stylet bearing/300 ml soil, number of females /5 g of roots and number of second stage juveniles /5 g roots were recorded by the formula according to Saxena and Singh (1987).

Results and discussion

A survey of different locations of important citrus growing districts i.e. Faisalabad, Sargodha, Layyah and T.T. Singh districts were conducted, and five orchards were selected at random at each location, disease prevalence, disease incidence and percent disease index was calculated

for the above said citrus growing areas.

Figure 1A shows disease prevalence in Sargodha, Bhalwal, Kot Momin, Silawali and T.T. Singh, which was 85.98%, 84.64%, 82.88%, 81.79% and 79.23%, respectively. Almost four out of five orchards visited were infected with dieback disease. Faisalabad and Layyah showed prevalence of 71.94% and 66.10%, respectively. Figure 1B shows disease incidence of 5.02 % in Kot Momin, 4.05 % in Bhalwal, 3.34 % in T.T. Singh and 3.35 % in Sargodha. It was low at Silanwali (2.31%), Faisalabad (2.13%) and Layyah (1.58%). Figure 1C shows maximum PDI of 35.18 %, 32.1 %, 31.81 % and 31.19 % respectively, in Bhalwal, T.T. Singh, Sargodha and Kot Momin while Silanwali, Layyah, and Faisalabad showed PDI of 29.03 %, 27.26 % and 25.12 % respectively.

Colonization percentage of the *Colletotrichum gloeosporioides* was found to be highest (42.68 %) in collected samples. *Colletotrichum acutatum* (14.04 %) was the second frequent fungus followed by *Lasiodiplodia theobromae* (8.42 %) and *Phomopsis citri Fawcetti* (6.87 %), respectively. All of these fungi were isolated abundantly from dying back branches. Colonization percentage of *Aspergillus niger* (4.76 %) and *A. flavus* (3.36 %) was minimum as compared to other isolated fungi.

Similar survey for decline and dieback was also carried out by Al-Azzeh *et al*. (2005) who isolated the citrus nematodes and calculated the disease incidence. Present results regarding the nematode population are also in conformity with them. Huang *et al*. (2013) also conducted an experiment and found that colonization percentage of *C. gloeosporioides* was maximum in dyeing back twigs of citrus. Samples collected from Bhalwal showed maximum abundance of *C. gloeosporioides* followed by Sargodha, Kot Momin, T.T Singh, Silanwali, Faisalabad and Layyah. Ramos *et al*. (2016) conducted a field survey of the main citrus growing areas in Portugal to assess the pathogenicity of *Colletotrichum* spp. along with morphological and genetic variability characterization. *C. gloeosporioides* was isolated from symptoms on fruits, twigs, leaves and flowers of many citrus cultivars. Results showed that frequency of *C. gloeosporioides* was high overall (87 %) than other isolated fungi from dieback and anthracnose of lemon in specific geographic locations.

Table I shows results of survey of different visited locations. The total number of nematode/300 ml soil recorded from visited locations was maximum in Bhalwal (3814.8) followed by Sargodha (3742.40). Nematode population in some of the visited orchards was above the economic threshold level. Mean comparison of citrus nematodes/300 ml soil from inspected five orchards from each location were recorded maximum in Bhalwal (3643.00) followed by Kot Momin (3586.40) and Sargodha (3573.60). Number of *Paratylenchus*/300 ml soil was maximum in Sargodha (103.20) followed by Bhalwal (101.60). Mean comparison of stylet bearing and non-stylet bearing nematodes/300 ml soil were also assessed and results indicated that stylet bearing nematodes are maximum in Kot Momin (55.00) while non-stylet bearing nematodes were maximum in T.T Singh (20.40). The mean comparison number of females of *T. semipenetrans* /5 g of roots was highest in Bhalwal (1196.60) followed by Sargodha (1128.20). Number of J2/5 g of roots were maximum in Bhalwal (14800) followed by Sargodha (13939) while it was minimum in Faisalabad.

AI-Azzeh and Abu-Gharbieh (2004) conducted a survey of citrus growing areas of Northern, Southern, and Central Jordan Valley and collected different isolates of *T. semipenetrans*. They also performed pathogenicity test in which sour orange seedlings were inoculated with different inoculum level of *T. semipenetrans* to access the damage threshold, pathogenicity and race identity. Results of pathogenicity tests showed that by inoculating 500 juveniles/pot, did not cause any significant reduction in plant growth parameters but inoculation of 1000 to 20,000 juveniles/pot resulted in significant reduction in vegetative growth of seedlings by 9.1% to 30.3%, while the root weight was reduced by 9.7 % to 30.9 % and showed symptoms of severe decline and dieback.

Results of this survey showed that there is maximum nematode population in Bhalwal followed by Kot Momin. The highest population of females and number of

Table I. Nematode population in different citrus growing areas.

Locations	Total nematode/300 ml soil	Citrus nematodes/300 ml soil	Paratylenchus/300 ml soil	Stylet bearing/300 ml soil	Non-stylet bearing/300 ml soil	No of females/ 5 g of roots	No. of J_2/ 5 g roots
Sargodha	3742.40a	3573.60a	103.20a	51.60b	12.20c	1128.20ab	13939ab
Silanwali	3450.20b	3303.20b	87.00b	49.60b	13.20c	1034.20cd	11974c
Kot Momin	3726.60a	3586.40a	74.60d	55.00a	11.40c	1063.40bc	12174c
Bhalwal	3814.80a	3643.00a	101.60a	52.00b	19.60a	1196.60a	14800a
Layyah	3197.00c	3070.00c	67.00e	42.80d	16.40b	951.60d	11987c
T.T Singh	3429.00b	3278.00b	82.00c	44.40cd	20.40a	1037.20bcd	13812b
Faisalabad	3127.20c	2950.80d	76.80d	45.80c	11.20c	985.80cd	10980d

juveniles of *T. semipenetrans* was recorded in Bhalwal. Sargodha, Silanwali, Layyah, T.T Singh and Faisalabad were also infested with citrus nematode. In Pakistan it was reported at Faisalabad in 1962 (Brown, 1962). Results regarding the citrus nematode population in Punjab province are in conformity with those of Iqbal *et al*. (2006).

The present survey confirms the occurrence of fungal pathogens mainly *C. gloeosporioides* and citrus nematode in District Sargodha, Layyah, T.T Singh and Faisalabad and association of these pathogens with citrus dieback disease.

Statement of conflict of interset

The authors have declared no conflict of interset.

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