Pak. J. Agri. Sci., Vol. 58(4),1291-1299;2021
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/21.399
http://www.pakjas.com.pk

Synergism of citrus nematode (*Tylenchulus semipenetrans* Cobb.) and *Colletotrichum gloeosporioides* plays a major role in citrus dieback

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Citrus is known as a major fruit due to its high nutritional value and adaptability in tropical and sub-tropical regions. Among diseases, citrus dieback is one of the most threatening diseases in which overall plant growth is reduced. Major causes are *Colletotrichum gloeosporioides* and citrus nematode (*Tylenchulus semipenetrans*) with 15-35% losses all over the world. Plant material was established adopting sanitary measures in earthen pots (12-inch diameter). Fresh culture of *C. gloeosporioides* and *T. semipenetrans* was prepared for the inoculation by following the standard procedures. In the first set, rootstocks were inoculated with 200mL of water having spore suspension of *C. gloeosporioides* while in in the second set, inoculation of *T. semipenetrans* was done by using 45 mL of water suspension having 2000 freshly hatched juveniles per pot, while at the rate of 1×10^7 spores/mL per plant. In the third set, the interaction of *C. gloeosporioides* and *T. semipenetrans* was studied by inoculating selected citrus rootstocks by both pathogens. After four months of inoculation, data were recorded on plant disease index (PDI) along with plant growth parameters (root weight, shoot weight, shoot weight, shoot length and number of leaves). Trifoliate orange and cox mandarin hybrid showed resistance against the development of *T. semipenetrans* while rough lemon, C-35 Citrange and sour orange were found susceptible. There was a significant difference in plant growth parameters between inoculated and healthy plants. Root weight and shoot weight decreased by 8.98g and 11.53g, while root length and shoot length decreased by 7.29cm and 13.5cm respectively as compared to control treatments in most susceptible rootstocks. Per cent Branch Infection (PBI) and per cent Disease Index (PDI) were maximum (71.52, 37) per cent on rough lemon respectively. Results regarding combined inoculation of *C. gloeosporioides* and *T. semipenetrans* showed that there was a significant difference in plant growth parameters between inoculated and healthy plants. In rough lemon, Root weight and shoot weight decreased by 13.86 and 20.57g respectively in diseased and healthy plants. Root length and shoot length decreased by 8.37 and 20.04cm respectively as compared to control treatments in most susceptible rootstocks. Overall results depicted that inoculation of both pathogens reduced plant growth more severely as compared to their individual application.
Keywords: Dieback, citrus, plant growth, synergism, citrus nematode.

INTRODUCTION

Citrus (*Citrus medica*) has a great socio-economic and nutritional impact on society in tropical as well as sub-tropical regions (Sidana *et al.,* 2013). In Pakistan, Punjab province supplies high-quality citrus and production-wise Kinnow contributes almost 86%, Musambi covers 10%, Feutral (4%) and Red Blush (1%) in citrus production (Imran & Hajan, 2020). Pakistan has the potential to produce 12-15 tons per hectare, but the actual yield is much lesser than the potential yield. It is due to multiple factors that are responsible for a big difference between its average and potential yield (Niaz *et al.,* 2004; Mahmood & Sheikh, 2006; Naseer, 2010). Dieback caused by *Colletotrichum gloeosporioides* is one of the severe citrus diseases in Pakistan (Rawal and Saxana, 1997; Naqvi, 2000; Alam, 2003; Yesmin *et al.,* 2017). Dieback leads to the sudden death of the plant from top to bottom due to more than one pathogenic factor (Ghosh, 1985). Dieback is a crop limiting factor and emerging as a serious issue of the citrus crop in most countries (Rawal & Saxana, 1997; Alam, 2003). The drastic percentage of citrus dieback disease on Kagzi lime and Elachi lemon recorded about 100% and 89.9%, respectively by Miah and Fakir (1988).

C. gloeosporioides is an airborne disease that causes dieback and wither-tip disease in citrus through toxic metabolites (Sharma and Sharma, 1969; Benyahia *et al.,* 2003; Huang *et al.,* 2013). Citrus nematode has been detected in declined citrus with dieback symptoms like yellowing, wilting,

Afzal, M.U.1, S.A. Khan, N. Javid and A. U. Malik. 2021. Synergism of citrus nematode (*Tylenchulus semipenetrans* Cobb.) and *Colletotrichum gloeosporioides* plays a major role in citrus dieback. Pak. J. Agri. Sci. 58:1291-1299.
[Received 24 Mar 2021; Accepted 2 Jul 2021; Published (online) 21 Sep 2021]

defoliation and death of twigs and branches. A complex of fungi and nematode results in plant wilting. Roots of citrus crop become rotten and dirty and failed to absorb nutrients and water from the soil (Bassanezi *et al.,* 2003; Spina, 2008). Symptoms of twig dieback caused by *C. gloeosporioides* were often observed on citrus trees (*Citrus sinensis*) advancing slowly. Wilting, yellowing and dropping of leaves occurred. Twigs turned dried having slightly raised, brown to black clumped pustules. (Benyahia *et al.,* 2003; Timmer *et al.,* 2000; Lima *et al.,* 2011).

In Pakistan, citrus nematode *(Tylenchulus semipenetrans)* has been reported from all the major citrus growing areas with varying degrees of infestations (Iqbal *et al.,* 2006; Mukhtar *et al.,* 2007; Khanzada *et al.,* 2008).

The physiological functions of plant nutrients have been understood well, but there are still unanswered questions regarding the dynamic interaction between nutrients and the plant-pathogen system (Huber, 1996). Citrus nematode attacks the root system of citrus plants and alter the physiology of roots. As the result, nutrient and water supply is reduced due to dysfunctional roots and nutritionally weak citrus plants become susceptible to the attack of other pathogens. *C. gloeosporioides* is an air-born fungal pathogen and attacks aggressively on the nutritionally weak citrus plant. This synergetic role of *T. semipenetrans* and *C. gloeosporioides* reduces the overall plant growth and increases the magnitude of dieback. The objective of the study was to find out the synergetic effect of Citrus Nematode (*T. semipenetrans*) and *C. gloeosporioides* for the development of citrus dieback. There is no work reported addressing the synergism of *T. semipenetrans* and *C. gloeosporioides* for the citrus dieback development.

MATERIALS AND METHODS

Establishment of plant material**:** Disease-free plant material was maintained at Citrus Nursery, University of Agriculture, Faisalabad. Healthy rootstocks (Rough lemon (*Citrus jambhiri*), C-35 Citrange (*Poncirus trifoliate* X *Citrus sinensis*), Cleopatra (*Citrus reshni*), Sour orange (*Citrus* X *aurantium),* Cox mandarin hybrid (*Scarlet mandarin* X *Trifoliate orange*), Carrizo citrange (*Citrus* X *insitorum*) and Trifoliate orange (*Poncirus trifoliata*), were further tested for fungi and nematode association to ensure disease-free.

All the pot experiments were done by using formalin sterilized sandy loam soil (8% clay, 17% silt and 72% sand) in earthen pots (12 inches diameter). Care was done to remove the formalin residues before filling pots (Atif *et al.,* 2015). Plants were watered regularly.

Sampling, isolation, purification, identification and preparation of spore suspension of C. gloeosporioides: Sampling was done by collecting citrus dieback affected twigs from the Sargodha region (32.0740° N, 72.6861° E). Isolation of fungal pathogens from collected samples was done by the procedure described by Ricker and Ricker, (1936). Purification and identification of colonized fungi on these pieces were done by the hyphal tip method on Potato dextrose agar (Ellis, 1971). Identification of purified fungal cultures was done on a morphological basis (Barnett and Hunter, 1998).

Pure cultures were further multiplied on PDA plates and incubation was done for seven days. Then produced conidia and mycelia were collected and transferred into a flask having 5 mL of Triton X-1000 (5%) and 500mL sterilized water and the flask was shaken vigorously for 10-12 minutes with the help of a mechanical shaker. After shaking, the suspension was passed by a double layer of cheesecloth. The concentration of these spore suspensions was adjusted to 1×10^7 spores/mL using Hirschmann hemocytometer (Sivakumar *et al.,* 1997).

Sampling, isolation and identification of T. semipenetrans**:** Feeder roots along with soil samples were processed for nematode isolation by using different techniques (Baermann funnel method described by McKenry and Roberts, (1985) and Whitehead and Hemming tray method given by Whitehead and Hemming, 1965). Identification was made on morphological features (Inserra *et al.,* 1988). After isolation, *T. semipenetrans* was multiplied on seven-month-old *Citrus jambhiri* in pots filled with soil mixture having an equal ratio of sand, silt and clay

Response of different rootstocks against T. semipenetrans and C. gloeosporioides**:** Citrus rootstocks (Rough lemon, C-35 Citrange, Cleopatra, Sour orange, Cox mandarin hybrid, Carrizo citrange and Trifoliata orange) were screened against *T. semipenetrans, C. gloeosporioides* and their combination to assess the effect of these pathogens on plant growth and disease severity.

A screening experiment was conducted in three sets. There were three replications of set and experiment was conducted under CRD. In the first set screening of rootstocks of citrus was done against *C. gloeosporioides* in earthen pots (12 inch diameter) in greenhouse. Established plants of selected rootstocks were inoculated by freshly prepared spore suspension of *C. gloeosporioides* by foliar application with the help of a hand sprayer. The rootstocks were inoculated with 200 ml of water having spore suspension of *C. gloeosporioides* at a rate of 1×10^7 spores/mL per plant. There was a control treatment for each rootstock sprayed with distilled water. The pots were irrigated regularly with tap water carefully. After four months of inoculation, data was recorded on disease parameters (total number of branches/plants, infected number of branches /plant, per cent branch infection /plant and plant disease index (PDI) along with plant growth parameters including root weight, shoot weight, root length, shoot length and number of leaves. Per cent disease index (PDI) and percent branch infection /plant were calculated by the given formulas respectively;

$$\text{Per cent disease index (\%)} = \frac{\text{Total sum of numerical ratings}}{\text{(No of obs) x (Maximum grade in ratting scale)}} \times 100$$

(Abedin and Chowdhury, 1982)

$$\text{Percent Brach Infection (\%)} = \frac{\text{Total sum of Infected branches}}{\text{Total branches}} \times 100$$

In the second set, selected rootstocks of citrus were screened out against *T. semipenetrans* under the same planting conditions as used in the first set. There were three replications of second set. Freshly hatched 2nd stage juveniles were counted by using a counting dish. Plants were inoculated with the freshly hatched second-stage juvenile of *T. semipenetrans* by soil application. For this purpose, in the middle of pots, 4-6 holes were made near the plant root system and pointed wood was used to make holes. Inoculation of *T. semipenetrans* was done by using 45mL of water suspension having 2000 freshly hatched juveniles per pot. Nematode suspension was poured in each hole equally in all pots and then pots were covered by polythene to avoid drying. Overwatering was avoided to prevent loss of nematodes through leaching. After four months of inoculation, data was recorded on plant growth parameters including root weight, shoot weight, root length, shoot length and number of leaves, while citrus nematode (*T. semipenetrans*) population (J_2/Root System, J_2/100mL soil, J_3/Root System, J_4/Root System and adults/Root System) were also recorded.

In third set interaction of *C. gloeosporioides* and *T. semipenetrans* was studied by inoculating selected citrus rootstocks by both pathogens. There were three replications of set three. Inoculation of *C. gloeosporioides* and *T. semipenetrans* was performed similarly as in the first and second sets respectively on the same plants. After four months of inoculation, data was recorded on plant disease index (PDI) along with plant growth parameters (root weight, shoot weight, shoot length and number of leaves.

Statistical analysis: Experiments were conducted using a completely randomized design. All experiments were performed thrice. The collected data were statistically analyzed using Statistix® 8.1 software.

RESULTS

Screening of different citrus rootstocks against C. gloeosporioides (percent branch infection and percent disease index): Fig 1. shows that Percent Branch Infection (PBI) was recorded maximum on Rough lemon (71.52) per cent followed by C-35 Citrange, Sour orange and Cleopatra (65.60, 53.21, 43.13) per cent respectively while minimum on Trifoliata orange (24.45) per cent followed by Carrizo citrange (37.75) per cent and Cox mandarin Hybrid (29.192) per cent. Percent Disease Index (PDI) was recorded maximum on Rough lemon (37) per cent followed by C-35 Citrange, Sour orange and Cleopatra (21, 32, 26) per cent respectively while minimum on Trifoliata orange (9) per cent followed by Cox mandarin Hybrid (11) per cent and Carrizo citrange (15) per cent.

Screening of different citrus rootstocks against citrus nematode (T. semipenetrans):

Parameters related to citrus nematode (T. semipenetrans) population (J_2/root system, J_2/100mL soil, J_3/root system, J_4/root system and adults/root system): A significant difference ($P \leq 0.05$) in population density of J_2 per root system was observed in different rootstocks screened out. Maximum population density of J_2 per root system was recorded in Rough lemon (10741) followed by C-35 Citrange, Cleopatra, Sour orange, Cox mandarin hybrid (8916, 6840, 5662, 4496) respectively, while minimum in Trifoliate orange followed by Carrizo citrange (981, 3177) respectively as shown in Fig 2a.

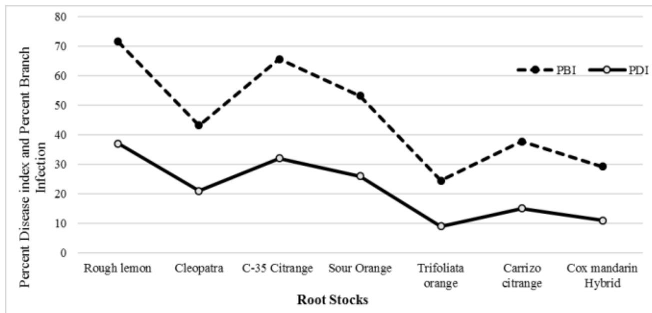

Figure 1. Screening of different citrus rootstocks against *Colletotrichum gloeosporioides* (Percent Branch Infection and Percent Disease Index)

Count of J_2 per 100mL soil was observed significantly different ($P \leq 0.05$) in different rootstocks screened out. Maximum population density of J_2 per 100mL soil was recorded in Rough lemon (1351) followed by C-35 Citrange, Cleopatra, Sour orange, Cox mandarin hybrid (1068, 826, 654, 513) respectively, while minimum in Trifoliate orange followed by Carrizo citrange (107, 321) respectively as shown in the Fig 2b.

A significant difference ($P \leq 0.05$) in population density of J_3 per root system was observed in different rootstocks screened out. Maximum population density of J_3 per root system was recorded in Rough lemon (94) followed by C-35 Citrange, Cleopatra, Sour orange, Cox mandarin hybrid (76, 64, 48, 40) respectively, while minimum in Trifoliate orange followed by Carrizo citrange (9, 31) respectively as shown in the Fig 2c.

A significant difference ($P \leq 0.05$) in population density of J_4 per root system was observed in different rootstocks screened out. Maximum population density of J_4 per root system was recorded in Rough lemon (467) followed by C-35 Citrange, Cleopatra, Sour orange, Cox mandarin hybrid (387, 318, 266, 219) respectively, while minimum in Trifoliate orange followed by Carrizo citrange (65, 139) respectively as shown in the Fig 2d.

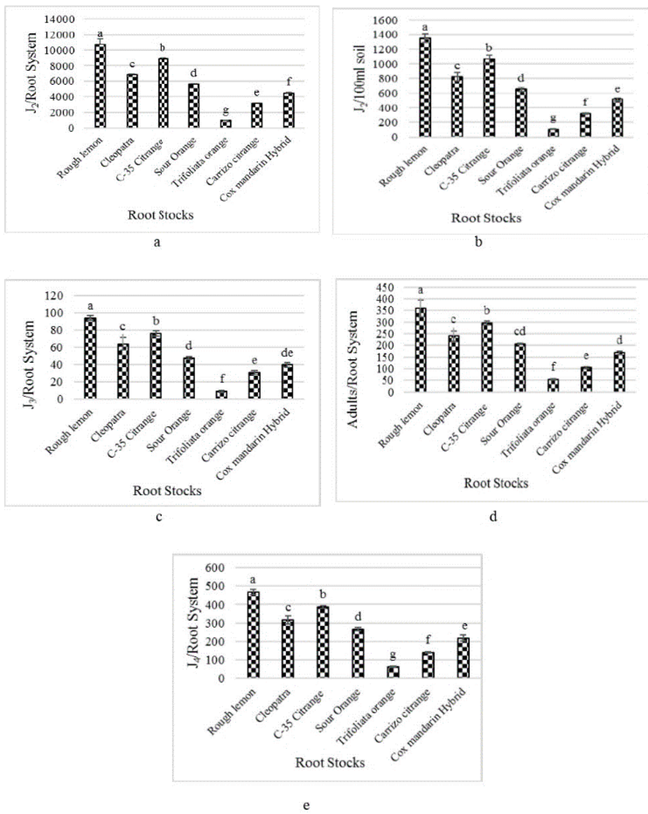

Figure 2. Response of different rootstocks against the development of *T.* semipenetrans (a: J_2/Root System, b: J_2/100mL soil, c: J_3/Root System, d: J_4/Root System, e: Adults/Root System). Means with in a column sharing the same letter are not significantly different from each other at P = 0.05 according to Bartlett's test.

Response of different rootstocks shows a significant difference in the development of adults per root system. Maximum population density of *T. semipenetrans* adults per root system was recorded in Rough lemon (358) followed by C-35 Citrange, Cleopatra, Sour orange, Cox mandarin hybrid (296, 240, 206, 168) respectively, while minimum in Trifoliate orange followed by Carrizo citrange (54, 104) respectively as shown in the Fig 2e. All the results were significantly different from each other *(P=0.05)*.

Screening of different Citrus rootstocks against T. semipenetrans (plant growth parameters): Table 1 shows the reduction in growth parameters of each citrus rootstock compared with its healthy control at P = 0.05. Root weight was recorded 18.484 g in rough lemon diseased plants while it was 27.46 g in healthy rough lemon. Root weight in healthy C-35 Citrange, Sour orange, Cleopatra, Carrizo citrange, cox mandarin hybrid and trifoliate orange was 25.58g, 26.47g, 23.52g, 26.58g, 29.53g and 30.83g while was 17.27g, 20.85g, 17.60g, 21.54g, 25.90g and 27.71g in diseased plants respectively. Maximum reduction in root weight was observed in rough lemon (8.98g) followed by C-35 Citrange, Sour orange, Cleopatra and Carrizo citrange respectively (8.30g, 5.61g, 5.92g, 5.04g) while the root weight reduced minimum in trifoliate orange (3.12g) followed by cox mandarin hybrid (3.62g).

Root length was recorded 37.64cm in rough lemon diseased plants while it was 44.93cm in healthy rough lemon. Root length in healthy C-35 Citrange, Sour orange, Cleopatra, Carrizo citrange, Cox mandarin hybrid and Trifoliate orange was 40.86cm, 37.08cm, 35.60cm, 39.85cm, 42.67cm and 46.68cm, while was 35.58cm, 32.39cm, 31.06cm, 35.56cm, 39.82cm and 44.16cm in diseased plants respectively. Root length was reduced significantly (P = 0.05) in rough lemon (7.19cm) followed by C-35 Citrange, Sour orange, Cleopatra

Table 1. Screening of different citrus rootstocks against *T. semipenetrans* (plant growth parameters).

Rootstock	Healthy /Diseased	Root weight (g)	Root length (cm)	Shoot weight (g)	Shoot length (cm)	No. of leaves
Rough lemon	Healthy	27.46bc	44.93ab	80.81bcd	84.48ab	123a
	Diseased	18.48gh	37.64ef	69.28j	70.98efg	102g
C-35 Citrange	Healthy	25.58cd	40.86cd	78.77def	80.90cd	112c
	Diseased	17.27h	35.58f	71.50ij	69.43fg	94h
Sour orange	Healthy	26.47c	37.08f	76.24fg	78.04d	108de
	Diseased	20.85fg	32.39g	71.88ij	69.16g	93hi
Cleopatra	Healthy	23.52de	35.60f	77.21efg	79.72cd	103fg
	Diseased	17.60h	31.06g	72.58hi	74.08e	90i
Carrizo citrange	Healthy	26.58c	39.85de	79.72cde	72.66ef	116b
	Diseased	21.54ef	35.56f	75.39gh	67.82g	106ef
Cox mandarin Hybrid	Healthy	29.53ab	42.67bc	83.40ab	82.66bc	119b
	Diseased	25.90cd	39.82de	79.75cde	79.74cd	111cd
Trifoliata orange	Healthy	30.834a	46.68a	84.93a	87.32a	126a
	Diseased	27.71bc	44.16ab	82.42abc	82.54bc	123a
LSD	-	2.47	2.70	3.06	3.33	3.39

Means within a column sharing the same letter are not significantly different from each other at P = 0.05 according to Bartlett's test and Carrizo citrange respectively (5.35cm, 4.68cm, 4.54cm, 4.29cm) and was reduced non-significantly in trifoliate orange and cox mandarin hybrid respectively (2.85cm, 2.52cm).

Shoot weight was recorded 69.28g in rough lemon diseased plants while it was 80.81g in healthy rough lemon. Shoot weight in healthy C-35 Citrange, Sour orange, Cleopatra, Carrizo citrange, cox mandarin hybrid and trifoliate orange was 78.77, 76.24g, 77.21g, 79.72g, 83.40g and 84.93g while was71.50g, 71.88g, 72.58g, 75.39g, 79.75g and 82.42g in diseased plants respectively. Maximum reduction in shoot weight was observed in rough lemon (11.53g) followed by C-35 Citrange, Sour orange, Cleopatra and Carrizo citrange respectively (7.27g, 4.36g, 4.63g, 4.33g) while the shoot weight reduced minimum in trifoliate orange (2.50g) followed by cox mandarin hybrid (3.65g).

Shoot length was recorded 70.98cm in rough lemon diseased plants while it was 84.48cm in healthy rough lemon. Shoot length in healthy C-35 Citrange, Sour orange, Cleopatra, Carrizo citrange, cox mandarin hybrid and trifoliate orange was 80.90cm, 78.04cm, 79.72cm, 72.66cm, 82.66cm and 87.32cm while was 69.43cm, 69.16cm, 74.08cm, 67.82cm, 79.74cm and 82.54cm in diseased plants respectively. Shoot length was reduced significantly in rough lemon (13.5cm) followed by C-35 Citrange, Sour orange, Cleopatra and Carrizo citrange respectively (11.46cm, 8.88cm, 5.64cm and 4.84cm) and was reduced non-significantly in trifoliate orange and cox mandarin hybrid respectively (4.78cm and 2.92cm).

The number of leaves per plant were recorded 102 in rough lemon diseased plants while 123 in healthy rough lemon. The number of leaves per plant in healthy C-35 Citrange, Sour orange, Cleopatra, Carrizo citrange, cox mandarin hybrid and trifoliate orange were 112, 108, 103, 116, 119 and 126 while, were 94, 93, 90, 106, 111 and 126 in diseased plants respectively. The number of leaves reduced significantly in rough lemon (21) followed by C-35 Citrange, Sour orange, Cleopatra and Carrizo citrange respectively (18, 15, 13, 10) while reduced non-significantly in trifoliate orange and cox mandarin hybrid respectively (3, 8).

Synergistic effect of citrus nematode (T. semipenetrans) + C. gloeosporioides for dieback: Table 2 shows the reduction in growth parameters of each citrus rootstock compared with its healthy control (α 5%). Root weight was recorded 14.28g in rough lemon diseased plants while it was 28.14g in healthy rough lemon. Root weight in healthy C-35 Citrange, Sour orange, Cleopatra, Carrizo citrange, cox mandarin hybrid and trifoliate orange was 24.18g, 26.31g, 24.48g, 27.18g, 31.23g and 32.66g while was 14.67g, 15.55g, 15.34g, 20.84g, 25.80g and 28.56g in diseased plants respectively. Maximum reduction in root weight was observed in rough lemon (13.86g) followed by Sour orange, C-35 Citrange and Cleopatra respectively (10.76g, 9.50g and 9.13g) while the root weight reduced minimum in trifoliate orange (4.10g) followed by cox mandarin hybrid and Carrizo citrange (5.43g) and (6.34) respectively. Root length was recorded 37.44cm in rough lemon diseased plants while it was 45.81cm in healthy rough lemon. Root length in healthy C-35 Citrange, Sour orange, Cleopatra, Carrizo citrange, cox mandarin hybrid and trifoliate orange was 42.29cm,35.42cm, 36.15cm, 36.82cm, 42.81cm and 47.26cm while was 35.98cm, 28.59cm,28.46cm, 32.04cm, 38.80cm and 43.70cm in diseased plants respectively. Root length was reduced significantly in rough lemon (8.37cm) followed by Cleopatra, Sour orange and C-35 Citrange respectively (7.69cm, 6.82cm, 6.31cm) and was reduced non-significantly in trifoliate orange (3.56 cm) followed by cox mandarin hybrid and Carrizo citrange respectively (4.01cm, 4.78cm).

Table 2. Synergistic effect of citrus nematode (*T. semipenetrans*) + *C. gloeosporioides* for dieback.

Rootstock	Healthy /Diseased	Root weight (g)	Root length (cm)	Shoot weight (g)	Shoot length (cm)	No. of leaves
Rough lemon	Healthy	28.14abc	45.81a	83.65abc	83.62abc	125ab
	Diseased	14.28f	37.44cde	63.08f	63.58de	95fg
C-35 Citrange	Healthy	24.18cd	42.29abc	79.27bc	82.50bc	115cd
	Diseased	14.67f	35.98de	65.30ef	67.83de	90gh
Sour orange	Healthy	26.31bcd	35.42de	78.22cd	77.56c	111de
	Diseased	15.55ef	28.59f	62.68f	66.36de	88gh
Cleopatra	Healthy	24.48cd	36.15de	78.17cd	78.76bc	104ef
	Diseased	15.34ef	28.46f	67.58ef	68.88de	84h
Carrizo citrange	Healthy	27.18abc	36.82de	77.67cd	70.30d	109de
	Diseased	20.84de	32.04ef	71.15de	62.66e	95fg
Cox mandarin Hybrid	Healthy	31.23ab	42.81abc	85.76ab	85.55ab	123bc
	Diseased	25.80bcd	38.80bcd	80.55abc	77.78c	112de
Trifoliate orange	Healthy	32.66a	47.26a	87.14a	89.58a	133a
	Diseased	28.56abc	43.70ab	82.47abc	82.68abc	125ab
HSD		5.94	5.40	7.13	7.02	9.40

Means within a column sharing the same letter are not significantly different from each other at P = 0.05 according to Bartlett's test

Shoot weight was recorded 63.08g in rough lemon diseased plants while it was 83.65g in healthy rough lemon. Shoot weight in healthy C-35 Citrange, Sour orange, Cleopatra, Carrizo citrange, cox mandarin hybrid and trifoliate orange was 79.27g, 78.22g, 78.17g, 77.67g, 85.76g and 87.14g while 65.30g,62.68g, 67.58g, 71.15g, 80.55g and 82.47g in diseased plants respectively. Maximum reduction in shoot weight was observed in rough lemon (20.57g) followed by Sour orange, C-35 Citrange and Cleopatra respectively (15.54g, 13.96g, 10.59) while the shoot weight reduced minimum in trifoliate orange (4.66g) followed by cox mandarin hybrid and Carrizo citrange respectively (6.52g, 5.21g). Shoot length was recorded 63.58cm in rough lemon diseased plants while it was 82.62cm in healthy rough lemon. Shoot length in healthy C-35 Citrange, Sour orange, Cleopatra, Carrizo citrange, cox mandarin hybrid and trifoliate orange was 82.50cm, 77.56cm, 78.76cm, 70.3cm, 85.55cm and 89.58cm while 67.83cm, 66.36cm, 68.88cm, 62.66cm, 77.78cm and 82.68cm in diseased plants respectively. Shoot length was reduced significantly in rough lemon (20.04cm) followed by C-35 Citrange, Sour orange and Cleopatra respectively (14.67cm, 11.20cm, 9.88cm) and was reduced non-significantly in trifoliate orange, Carrizo citrange and cox mandarin hybrid respectively (6.90cm, 7.64cm, 7.77cm).

The number of leaves per plant were recorded 95 in diseased plants while 125 in healthy rough lemon. The number of leaves per plant in healthy C-35 Citrange, Sour orange, Cleopatra, Carrizo citrange, cox mandarin hybrid and trifoliate orange was 115. 111, 104, 109, 123 and 133 and were 90, 88, 84, 95, 112 and 125 in diseased plants respectively. The number of leaves reduced significantly in rough lemon (30) followed by C-35 Citrange, Sour orange, Cleopatra and Carrizo citrange respectively (25, 23, 20, 14) while reduced non-significantly in trifoliate orange and cox mandarin hybrid respectively (8, 11).

DISCUSSION

Citrus dieback is the emerging threat to the citrus orchards because it does not cause only loss of fruit but the whole plant gradually dies and fruit yield, quality and number of plants per hectare starts to decrease. *T. semipenetrans* infect the citrus plants and suck the plant food and the efficiency of the plant root system is decreased, as a result, the infected plants become nutrient deficient and became susceptible to the attack of *C. gloeosporioides.* Therefore, by the involvement of *T. semipenetrans*, the fungal pathogen *C. gloeosporioides* infect the citrus plants more aggressively causing an increased magnitude of dieback.

Cultivation of tolerant or resistant cultivars could be used as an effective tool for the proper and environmentally safe management of diseases, in this way use of pesticides and other laborious management strategies could be eliminated. (Gebhardt and Valkonen, 2001). Plant resistance plays a key role in the management of disease-causing pathogens. In a particular region, it is hard to find such type of cultivars which are resistant to a wide range of pathogens, so it is need of time to discover most threatening and damaging pathogens in a region and find out suitable cultivars having resistance against them. (Pedley and Martin, 2003).

Seven available citrus rootstocks were screened out against the *T. semipenetrans.* All of the root stocks responded differently to the *T. semipenetrans* infestation. Nematode could not establish active feeding sites on the roots of resistant host plants as the result of hypersensitive responses, ultimately female reproduction is reduced. (Williamson and Kumar, 2006). Prasad *et al.*, (1997) claimed Rough lemon and Trifoliate orange as resistant rootstocks against *T. semipenetrans* but the results of the present study contradict this claim in the case of Rough lemon but were in accordance in case of Trifoliate orange. Rootstocks responding differently against *T. semipenetrans* shows that there is one foremost gene or a multiple tandem gene participating in the development of resistance against *T. semipenetrans.* (Ling *et al.*, 2000). Results of the present study were in line with studies of Hutchison, 1985; Verdejo-Lucas *et al.*2003 and Nazir *et al.* 2008.

Different crops react similarly to the invasion of different nematodes by changing oxidants and antioxidants. The rate of increase depends upon the host plant, nematode species and initial population. (Davis *et al.*, 2000; Huang *et al.*, 2004; Afifi *et al.*, 2014). The mode by which resistance is inherited is not understood. Further investigations are needed to understand the mechanism of resistance (Kaplan, 1981). Numerous mechanisms including the formation of wound periderm and hypersensitive response are involved in the resistant response. (Van Gundy and Kirpatrick, 1964; Kaplan and O'Bannon, 1981).

In the present investigation, it was also observed that the root system of citrus plants is affected due to semi penetration of *T. semipenetrans* and continues withdrawal of nutrients results in poor plant growth due to invasion of nematodes and development of feeding sites, the ability of root system to absorb water and nutrient is compromised. Minimum reduction in growth parameters in inoculated treatments as compared to uninoculated treatments was observed in Trifoliata orange and Cox mandarin Hybrid so both of these rootstocks show resistance against *T. semipenetrans,* while Rough lemon and C-35 Citrange show a maximum reduction in growth parameters and show susceptibility to *T. semipenetrans.* Ayazpour *et al.*, (2010) indicated during an investigation that inoculation of *T. semipenetrans* to the citrus plants results in decreased plant growth, plants facing nematode invasion show decreased root weight and shoot weight. Results of the present study were in conformity with Deka *et al.* (2003) and Montasser *et al.* (2012).

Citrus plants are often facing biotic and abiotic stresses screening is a high priority goal for the selection of resistant germplasm. This is of much importance for the citrus rootstocks which are severely damaged by the fungal pathogens. All the seven citrus rootstocks were screened out against *C. gloeosporioides* to assess the level of infection in terms of per cent disease index (PDI) and per cent branch infection (PBI). Results indicated that Trifoliata orange and Carrizo citrange showed resistance against infection of *C. gloeosporioides* while Rough lemon, C-35 Citrange and Sour orange show maximum infection exhibiting susceptibility. Screening of citrus rootstocks against *C. gloeosporioides* is not reported from previous literature. Although pathogenicity of *C. gloeosporioides* has been confirmed by Benyahia *et al.* 2003; Ajay 2014; Ramos *et al.* 2016 and Mayorquin *et al.* 2019.

Pathogen attack to healthy plant results in impaired physiology reduced nutrient uptake with less assimilation and translocation from the root system to the shoot system. (Marschner, 1995). Invading pathogens consume a significant amount of nutrients for self-establishment which results in a reduced availability of nutrients for the host plant and host susceptibility is increased due to nutrient deficiency (Timonin, 1965). In the present study, a screening experiment was conducted in which selected rootstocks of citrus were screened out against citrus nematode (*T. semipenetrans)* and *C. gloeosporioides* together. Results of revealed that when the plants were exposed to both pathogens at the same time, plant growth is affected with a greater magnitude as of plants exposed to *T. semipenetrans* and *C. gloeosporioides* individually. Trifoliata orange and Cox mandarin hybrid performed best and exhibit a minimum reduction in plant growth parameters in inoculated or diseased plants as compare to healthy plants. Rough lemon, C-35 Citrange and Sour orange indicated maximum susceptibility and show a maximum reduction in plant growth parameters in inoculated or diseased plants as compared to healthy plants. Both of the pathogens infecting citrus plants at the same time might have a synergetic relationship resulting in increased damage to the citrus plants. When the root system of citrus plants is invaded by the *T. semipenetrans*, a sufficient supply of water and nutrients is disturbed, so the nutrient-deficient plants might be more susceptible due to physiological changes and could not resist the fungal infection. Safdar *et al.* (2013) conducted an experiment in which both *Fusarium semitectum,* and *T. semipenetrans* were inoculated together and cause more plant growth reduction as compared to their individual application. During interaction, the nematode plays the primary role as a modifier of the host, making it more susceptible to other pathogens (Pitcher, 1978; Powell, 1979). Results of the present study confirmed the findings of different workers who worked on the synergism of fungi and plant-parasitic nematodes (Nicholson *et al.,* 1985; Melgar *et al.,* 1994; Yang and Rizvi, 1994; Roy *et al.,* 1997; Gao *et al.,* 2006).

***Conclusion*:** The herein reported studies were conducted to explore the synergetic role of *T. semipenetrans* and *C. gloeosporioides* for the development of citrus dieback. Different available citrus root stocks were tested against both pathogens individually and in combination. Results indicated that when both pathogens attack simultaneously, there is higher magnitude of dieback as compare to their individual attack.

***Acknowledgement*:** Financial support for this research work was provided by Higher education commission of Pakistan.

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