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Research Article

Confronting Dual Threats: Management of *Tylenchulus semipenetrans* Cobb and *Fusarium oxysporum* in Citrus Orchards causing Decline

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Abstract

Citrus decline is a multifaceted challenge affecting citrus orchards worldwide as well as Pakistan, poses a significant threat to the citrus industry. This decline is often attributed to the synergistic effects of various pathogens, *Fusarium oxysporum* and *Tylenchulus semipenetrans* emerging as key contributors. The present study was conducted during 2018-2021to investigate the different chemicals for the management of citrus decline under natural field conditions. The experiment was designed in RCBD with five replicates. Each treatment was applied twice a year at time of new flushes Data were collected each year before treatment application on the basis of no. of infected roots piece/ 100 pieces of roots for fungi, no. of juveniles/ 100 grams of soil and no. of females/ gram of roots for nematodes, and After six month of last application, as compared to pretreatment data, maximum percent inhibition of fungus, number of juveniles and number of females were found in T_1 89.67%, 89.87% & 90.39 followed by T_2 84.61%, 83.91% & 82.82%, T_3 79.54%, 88.17% & 89.45%, and T_4 76.78%, 81.92% & 83.04% respectively. While in the control treatment the no. of infected roots piece/ 100 pieces of roots (36.96%), no. of Juveniles/ 100 grams of soil (94.11%) and no. of females/ gram of roots (109.78%), percent increased many fold which caused citrus decline. All chemicals treatments reduced significantly in nematode and fungal infestations.

Keywords: Citrus, Decline, *Fusarium oxysporum*, Management, Nematicides, Nematode, *Tylenchulus semipenetrans*

Introduction

Citrus production stands as a prominent contributor in the global agricultural sector, ranking among the largest yields worldwide. In the realm of international trade, citrus holds the second position, trailing only bananas and surpassing apples by more than

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double in volume. Citrus has solidified its status as one of the most crucial fruits within 52 countries. Brazil and China emerge as the primary powerhouses in citrus production, leading the global pack (Mahmood *et al.*, 2014) Beyond its economic impact, citrus holds immense nutritional value, renowned for being a rich source of vitamin C, these fruits also boast a composition of 3-4% sugar and noteworthy amounts of essential minerals, i.e. calcium and magnesium. This nutritional profile adds increased appeal of citrus as a dietary staple for many individuals around the world. The success of citrus cultivation is particularly evident in regions characterized by tropical and sub-tropical conditions, where temperature fluctuates between 4 ^{0}C and 50 ^{0}C. Moreover, the soil in these areas provides a favorable environment conducive to robust citrus growth and high yields (Sidana *et al.*, 2013). In the sphere of citrus production, Pakistan holds a significant position, ranking among the top 15 citrus-producing countries globally (Mahmood *et al.*, 2014). The citrus cultivation landscape in Pakistan spans a total area of 448.88 thousand acres, resulting in an approximate production of 2468.67 thousand tons, as reported by (GOP, 2022-23). Punjab province in Pakistan emerges as a key contributor to the country's citrus industry, supplying high-quality citrus fruits. In terms of production Kinnow takes the lead (86%), followed by Musambi (10%), Feutral (4%) and Red Blush (1%) (Cheema and Jamali, 2020). In Pakistan to achieve a yield ranging from 12 to 15 tons per hectare, the actual citrus yield falls down significantly. The gap between the average and potential yield underscores the challenges faced in optimizing citrus production in the region (Niaz *et al.*, 2004; Naseer, 2010).

However, the potential for enhanced citrus production faces deterrents as the industry grapples with due to several diseases, such as canker, Phytophthora root rot, citrus decline/wilt, stem rot, vascular wilt, feeder-root rot, and dieback twig-blight pose considerable threats. Notably, citrus decline emerges as the most devastating disease affecting citrus production in Pakistan, (Safdar *et al.*, 2013). The diminution in citrus production is further compounded by plant diseases caused by soil-borne pathogens, specifically nematodes and fungi. These diseases are recognized as major challenges in global agricultural production (Abd-Elgawad and McSorley, 2009) The presence of soil-borne pathogens adds an additional layer of complexity to the management of citrus cultivation, necessitating a holistic approach to address the various factors contributing reduced citrus yields in Pakistan. As the global demand for citrus continues to rise, understanding the factors influencing its production becomes paramount. However, the citrus industry faces a significant challenge in the form of citrus decline, a complex syndrome affecting orchards worldwide.

Citrus decline is a pervasive threat to global citrus cultivation, manifests as a complex syndrome influenced by various biotic and abiotic factors. Among the numerous contributors to this decline, *Fusarium oxysporum* and *Tylenchulus semipenetrans* have emerged as key players, forming a formidable alliance that compromises the health and productivity of citrus orchards. In Pakistan, citrus nematode (*T. 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 intricate interplay between *F. oxysporum* and *T. semipenetrans* creates a unique set of

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challenges for citrus orchard management. *Fusarium oxysporum* exhibits a pathogenic behavior, causing vascular wilt and root rot, while *T. semipenetrans* exacerbates the issue by further weakening the plants root system through its parasitic feeding habits. This dual assault not only compromises the plants ability to absorb water and nutrients but also renders it more susceptible to secondary infections, intensifying the overall decline.

Effective management strategies are essential to mitigate the impact of both *F. oxysporum* and *T. semipenetrans* in citrus orchards. As the citrus industry plays a crucial role in global agriculture and economy, finding sustainable and integrated approaches to tackle this decline is of paramount importance. This introduction sets the stage for a comprehensive exploration of the management practices and innovative solutions that can be employed to safeguard citrus orchards against detrimental effects of *F. oxysporum* and *T. semipenetrans*, ensuring the resilience and productivity of this vital agricultural sector. Keeping in view the importance of citrus in the economy of Pakistan this experiment was designed to investigate the management of citrus decline.

Methodology

A survey was conducted during 2018 and selected 7 to 8-years-old citrus orchard showing10-15% decline symptoms. Root and soil samples were collected for the confirmation of fungus and nematodes attack on plants. Fibrous roots and soil samples were collected from 4 sides of plants to make composite sample. These samples were put in craft paper bags and labeled properly. These samples brought back Lab and placed in incubator at 6 ^{0}C for further studies.

Assessment of fungus

One gram of root sample from composite sample was washed carefully and dried on filter paper. After that root was cut into 100 pieces and surface sterilized by dipping in 70% NaOCl for 1 minute and washed twice with distilled water. Then these pieces were placed on PCNB medium for confirmation of *Fusarium* sp. The sampled plates were incubated at 25 ^{0}C $\pm$ 2 for 5 days. After fungus growth, picked and checked the fungal mycelium and spores by making slide under microspore for the confirmation of *F. oxysporum*. Also, roots pieces were counted which have fungal growth for pretreatment data of fungus.

Assessment of nematode

Hundred gm soil was weighed and processed by Baremann Funnel method. After 48 hour, water was collected in beaker and checked under Inverted microscope at 40X lens to count the no. of juveniles of citrus nematode. Two ml of water was poured in counting chamber and counted the juveniles. This process was repeated thrice to minimize the error for presence of total no. of juveniles in 100 gm of soil.

Number of females per gm of roots: one gram roots were washed carefully to avoid detachment of females. For counting the number of females, roots were stained with Acid fuchsin. The solution was made by mixing glycerol 40 ml, phenol 20 ml, lactic acid 20 ml, distilled water 20 ml and Acid fuchsin 15 mg. Roots were dipped in boiling solution for 1-3 minutes and the excessive solution contents were removed by rinsing in water. For de-staining, the roots were dipped in lacto phenol solution. Only females remained stained while roots were discolored after dipping in clearing solution and number of females were counted under the stereomicroscope. Pretreatment data on basis

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of number of females were counted on one gram of citrus roots.

The present study was planned with five treatments (**T_1**: Rugby 5G (Cadusafos) @ 50g/plant & Score (Difenoconazole 250 EC) @ 1ml/L of water; **T_2**: Rugby 5G (Cadusafos) @50G/Plant & Bordeaux Solution @ 4:4:50/plant; **T_3**: Furadan 3G (Carbofuran) @100g/plant & Score (Difenoconazole) 250 EC @ 1ml/L of water; **T_4**: Furadan 3G (Carbofuran) @100g/plant & Bordeaux Solution @ 4:4:50/plant and **T_5**: Control) under natural field condition. Each treatment was applied twice in year at time of new flushes of plants. The experiment was designed in RCBD with five replicates. Plants without chemical were kept as control. Data were collected each year before each treatment application on basis of no. of females/ gram of roots, no. of juveniles/ 100 grams of soil and no. of infected roots piece/ 100 pieces of roots.

Results and Discussion

Before applying treatments, 100 roots were analyzed and recorded average data of fungus for five treatments i.e. 36.8, 35.2, 31.2, 33.6 & 34.6 respectively. For nematodes, 100 gm soil and one gm roots were analyzed and recorded average data for number of juveniles and number of females of five treatments i.e. 1167.6, 1055.4, 1307.2, 1284.4 & 1213.9 and 887.4, 903.2, 817.6, 898.6 & 925.8 respectively (Table-1).

Table 1 Incidence of fungal and nematode on citrus (root and soil) before chemicals application.

Treatments	No. of infected roots/ 100 pieces of root	No. of Juveniles Per 100 grams of Soil	No. of Females Per Gram of Roots
T_1	36.8	1167.6	887.4
T_2	35.2	1055.4	903.2
T_3	31.2	1307.2	817.6
T_4	33.6	1284.4	898.6
T_5	34.6	1213.9	925.8

All four treatments gave better results as compared to control treatment. After 1st year treatments application, maximum inhibition of fungus, number of juveniles and number of females were found in $T_1$11.4, 282.4 and 239.2 followed by T_2 13.2, 288.2 and 246, T_3 15.4, 380.8 and 283.8 and T_4 16.2, 395.8 and 297.4 respectively as compared to control treatment 37.6, 1662.4 and 1197.6. After 2nd year treatment application, T_1 7.2, 217.6 and 181.6 was found the most effective treatment and T_4 11.2, 345.2 and 244.2 was least effective treatment, respectively. T_2 7.8, 229.4 and 193.4 and T_3 10.6, 327.6 and 221.4 showed intermediate results. Similar results were found after last application, Maximum inhibition of fungus, juveniles and females were found in T_1 3.8, 118.2 and 85.2 and minimum inhibition was found in T_4 7.8, 232.2 and 152.4 respectively whileT_2 and T_3 showed intermediate results (Table 2, 3 and 4).

After last application, the maximum percent decrease over control of fungi, juveniles and females were recorded (92.05%, 94.98% and 95.61%) by T_1 while T_4 showed the least percent decrease over control fungi, juveniles and females (83.68%, 90.15% and 92.15%) while all other treatments T_2 & T_3 (84.94%, 94.70% and 95.10%) and (89.96%, 91.08% and 92.77%) revealed intermediary results after last application, respectively (Figure 1, 2 & 3).

On the basis of maximum percent decrease over control of fungi, number of juveniles and number of females inhibition premeditated the R^2 Value ($R^2 = 0.992$, $R^2 = 0.9994$ & $R^2 = 0.9908$) and equations ($y = 11.185x + 59.077$, $y = 5.9857x + 77.112$ and $y = 7.7933x +$

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72.667) by T_1 while T_4 showed the minimum percent decreased over control of fungi, no. of juveniles and no. of females inhibition calculated R^2 value ($R^2 = 0.9871$, $R^2 = 0.9997$ and $R^2 = 0.9867$) and equations ($y = 13.384x + 44.414$, $y = 6.9775x + 69.141$ and $y = 8.4931x + 67.244$), respectively.

Table 2. Efficacy of fungicides for management of fungi under natural field conditions.

Treatments	(2018-19)	(2019-20)	(2020-21)
T1 (Rugby+Score)	11.4 c	7.2 c	3.8 c
T2(Rugby+Bordeaux solution)	13.2 c	7.8 c	4.8 c
T3(Furadan+ Score)	15.4 b	10.6 b	7.2 b
T4(Furadan+Bordeaux solution)	16.2 b	11.2 b	7.8 b
T5(Control)	37.6 a	41.4 a	47.8 a

Table 3 Efficacy of nematicides for management of juveniles under natural field conditions.

Treatments	(2018-19)	(2019-20)	(2020-21)
T1 (Rugby+Score)	282.4 c	217.6 d	118.2 d
T2(Rugby+Bordeaux solution)	288.2 c	229.4 d	124.8 d
T3(Furadan+ Score)	380.8 b	327.6 c	210.2 c
T4(Furadan+Bordeaux solution)	395.8 b	345.2 b	232.2 c
T5(Control)	1662.4 a	2024.8 a	2356.4 a

Table 4 Efficacy of nematicides for management of females under natural field conditions.

Treatments	(2018-19)	(2019-20)	(2020-21)
T1 (Rugby+Score)	239.2 c	181.6 e	85.2 d
T2(Rugby+Bordeaux solution)	246 c	193.4 d	95.2 d
T3(Furadan+ Score)	283.8 b	221.4 c	140.4 c
T4(Furadan+Bordeaux solution)	297.4 b	244.2 b	152.4 b
T5(Control)	1197.6 a	1669.2 a	1942.2 a

While all other treatments T_2 & T_3 R^2 value ($R^2 = 0.9886$, $R^2 = 1$ and $R^2 = 0.993$), equations ($y = 12.947x + 46.897$, $y = 6.0201x + 76.639$ & $y = 7.8197x + 72.018$) and R^2 value ($R^2 = 0.9713$, $R^2 = 0.9995$ and $R^2 = 0.9768$), equations ($y = 12.532x + 53.606$, $y = 6.9931x + 70.012$ and $y = 8.2342x + 68.801$) respectively, revealed intermediary results after last application.

After last application; as compared to pre and post treatment data, maximum percent inhibition of fungus, number of juveniles and number of females were found in T_1 89.67%, 89.87% and 90.39 followed by T_2 84.61%, 83.91% and 82.82%, T_3 79.54%, 88.17% and 89.45%, and T_4 76.78%, 81.92% and 83.04% respectively. While in the control treatment, no. of females/ gram of roots (109.78%), no. of Juveniles/ 100 grams of soil (94.11%) and no. of infected roots piece/ 100 pieces of roots (36.96%) percent increased many fold which caused citrus decline (Table 5).

Citrus decline poses a significant threat to orchards, causing not only a loss of fruit but also the gradual demise of the entire plant, resulting in a decrease in fruit yield, quality, and the number of plants per hectare. The majority of fibrous roots crucial for citrus health grown within the surface 24–30 inches of soil, decreased in abundance from the tree trunk to the row middle, emphasizing the importance of proper under-canopy

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management. Ensuring healthy citrus-propagating material is vital and these are encompassing the consideration of nematodes, fungi bacteria, viruses and also many infectious pathogens (Abd-Elgawad, 2016). An examination of infected parts of citrus trees revealed the association of fungi; *F. semitectum, F. solani, Phytophthora spp., Helminthosporium spp., A. flavus, A. niger,* and *Rhizopus* spp., with *F. semitectum* dominating the majority of selected samples. The prevalence of *Fusarium* species is influenced by climatic factors and they are found in various soil types (Latiffah and Baharuddin, 2007).

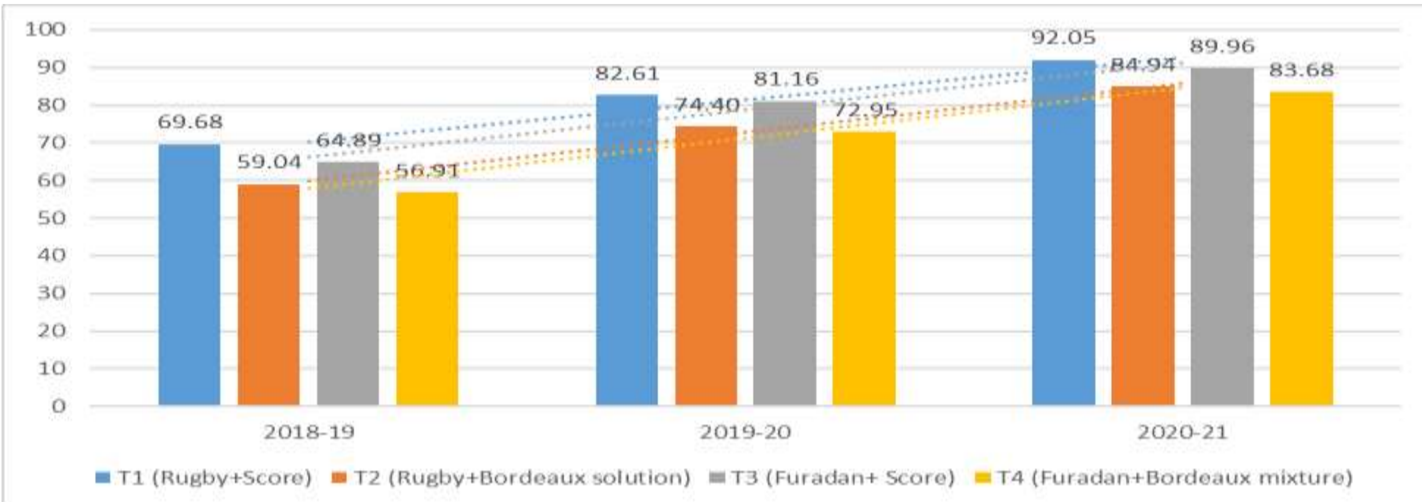

Figure 1. Percent decreased over control in infected root piece/ 100 piece of root.

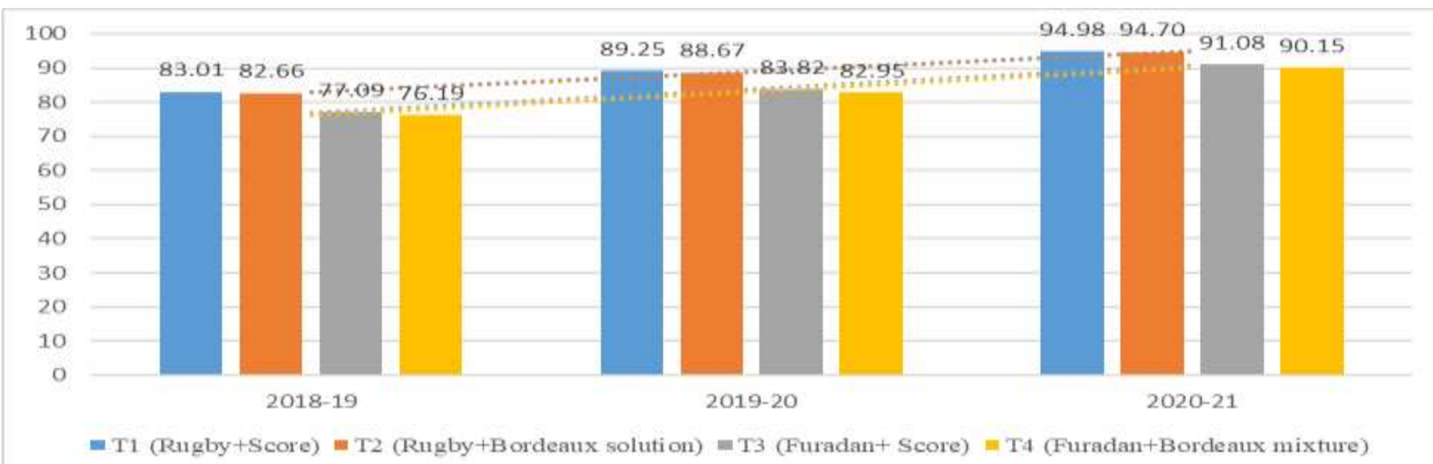

Figure 2. Percent decreased over juveniles per 100 grams of Soil.

Table 5. Comparison of pre and post treatments for percent decreased/increased after management of fungus and nematode.

Treatments	No. of infected root piece/100 piece of root	No. of Juveniles/100 grams of Soil	No. of Females/ Gram of Roots
T_1 (Rugby+Score)	89.67	89.87	90.39
T_2(Rugby+Bordeaux solution)	79.54	88.17	89.45
T_3(Furadan+ Score)	84.61	83.91	82.82
T_4(Furadan+Bordeaux solution)	76.78	81.92	83.04
T_5(Control)	36.96	94.11	109.78

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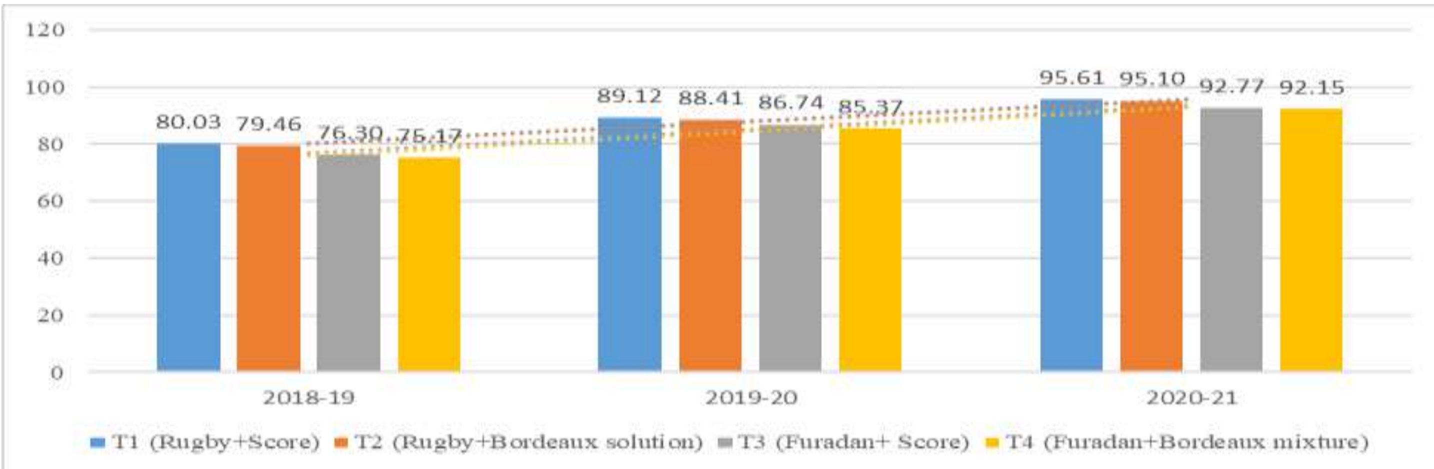

Figure 3. Percent decreased over females per gram of roots.

(Safdar *et al.*, 2013; Zaccardelli *et al.*, 2008) revealed that the interaction between *F. semitectum* and *T. semipenetrans* results in more significant plant growth reduction compared to their individual applications. *T. semipenetrans* plays a primary role as a modifier of the host during this interaction, increasing the plant's susceptibility for other pathogens (Pitcher, 1978; Powell, 1979). The nematode infects citrus plants, reducing the efficiency of the plant root system, making infected plants nutrient deficient and more susceptible to *F. oxysporum*. The involvement of *T. semipenetrans* intensifies the decline, allowing *F. oxysporum* to infect citrus plants more aggressively.

Effective management of nematodes in citrus involves various production practices such as tillage, solarization, and the addition of organic and inorganic amendments. Shokoohi and (Shokoohi and Duncan, 2018) emphasized the efficacy of pre-plant nematicides like metam sodium and 1,3-dichloropropene. Placement within the surface 24–30 inches of soil, targeting areas with the highest nematode density, is crucial for nematicide effectiveness. However, repeated applications may lead to diminished efficacy due to accelerated microbial degradation.

Various nematicides have successfully been used to decrease the population of *T. semipenetrans* in citrus. However, repeated applications are needed to maintain reduced densities and consistent yield increase. Two main groups of nematicides, oxime-carbamates (aldicarb, oxamyl, carbofuran) and organophosphates (fenamiphos, ethoprophos, and cadusaphos) are used for the management of nematodes. Granular nematicides has shown superior efficacy and Carbofuran is reported to inhibit cholinesterase in nematode, offering a non-fumigant alternative. (McClure and Schmitt, 1996) but the availability and profitability of nematicides vary (Shokoohi and Duncan, 2018; El-Tanany *et al.*, 2018). Biologicals and natural compounds, such as plant extracts, organic manure, fungi, bacteria, and EPN suspension, are explored as alternatives of chemical nematicides (Ibrahim *et al.*, 2019; Montasser *et al.*, 2012; Shehata *et al.*, 2021).

The reduction of number in females, juveniles and fungus after chemicals treatments, as found in our study, agrees with (Ahmed *et al.*, 2009; Shokoohi and Duncan, 2018) in combating this malady.

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Conflict of Interest

The authors have not declared any conflict of interest.

Authors Contributions

All the authors contributed equally in the manuscript.

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