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The Impact of Abiotic Factors on Soil-borne Pathogenic Fungi in Orchards

We examined fine roots and soil samples from different trees across 20 sites of orchards. Fungal frequency of occurrence (%) were calculated in fine roots and soil per location and across locations. Fusarium spp. was the most frequent fungi in fine roots in both Al Za'franiya (100%) and Tarmiyah (100%); and the most frequent fungi across locations with (84.2%). While Aspergillus spp. was the most frequent fungi in soil dilution in both Al Za'franiya (75.0%) and Abu Ghraib (50.0%); and the most frequent fungi across locations with (50.0%). Further, we examined five abiotic factors, irrigation, geographical locations, type of plant, type of soil, and type of water to identify their effect on fungal community of fine roots and soil in terms of least squares means and standard error (LS means±SE) across 20 orchard sites. Al-Za'franiya fine roots revealed abundance of fungal community (5.0±1.14), followed by Dora (1.0±0.93), Abu Ghraib (0.6±0.72), and Tarmiyah (0.3±0.51); while Lythraceae revealed a significant effect on fungal community (5.5±1.15). Similarly, Al-Za'franiya soil recorded a significant effect on fungal community (4.0±1.17); while Lythraceae had a significant effect on fungal community (5.0±1.10). Whereas irrigation, type of soil, and type of water showed no influence ($p > 0.05$) on fungal community in both fine roots and soil. These findings highlight the predominant of Fusarium spp. reflects the importance of this fungus as a potential pathogen in the agricultural environment of Iraq. Further, the variation in the effects of tested abiotic factors on the fungal community of soil-borne pathogenic fungi of both fine roots and soil emphasizes the epidemic risk on agricultural ecosystem in Iraq.

Keywords: Irrigation; Abiotic factors; Pathogenic fungi; Ecosystem
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1. Introduction

The relationship between tree composition and ecological variables such as climate factors, topographic factors, soil factors and human activity is considered a fateful issue in environment and agricultural sciences [1,2]. Orchards include trees, shrubs, grasses and wild flowers. Orchards have been recognized to grow food and provide a broad range of wildlife [3]. For example, the market of apple orchards recorded a value of $78.8 billion in 2019 [4]. Another example, 20% out of the total British Heteroptera and Auchenorrhyncha fauna were recorded in three orchards that located South-East England [5,6]. Recently, many Orchards suffered from new threats such as the chilling accumulation, extreme both temperatures low and high, floods, droughts, storms, intense winds, frost event, novel pests [7].

Soil-borne fungi have numerous pathogens responsible for worldwide plant diseases [8]. Pathogenic fungi infect essential crops and economically viable commodity crops that leads to a huge risk for the global food security [9]. The spread of these fungi can enhance this risk result to epidemics [10]. Soil borne fungi plays a critical role not only in plant population dynamics in natural ecosystems but also epidemics of plant diseases in agricultural ecosystems [11]. Numerous epidemics result by soil-borne fungi such as *Fusarium* spp., *Verticillium* spp., *Rhizoctonia solani* and *Gaeumannomyces graminis* based on the reaction between fungal growth and the temporal and spatial heterogeneity for environment of soil [11]. Essentially, abundance and diversity of soil-borne fungal community were reflected by soil physical properties (soil texture and structure) [12] and environmental factors (vegetation system and topography) [13].

The variation in soil conditions, such as moisture, pH, temperature, O_2, and physico-chemical properties, soil environments, such as salinity, metal toxicity, drought and submergence, and plant varieties along with agricultural practices may diverse both the composition and characteristics of soil microbes and plant susceptibility/resistance against these biotic and abiotic stresses/factors [14]. Further, abiotic stresses, especially drought, altered soil environments resulting in effecting both soil microbes and plants [15].

In Iraq, limited studies were conducted to understand the fungal community associated with plant roots [16] or soil [17,18], and the impact of some biotic and/or abiotic factors on pathogenic fungi [19,20], yet, there were no studies on how the irrigation, type of soil, and type of water may affect the fungal community of soil- borne pathogenic fungi

of fine roots and soil in different types of orchards in Baghdad. Thus, we firstly hypothesized that fungal community varies depending upon the type of samples (fine roots and/or soil), and secondly, that abiotic factors effect fungal community of soil-borne pathogenic fungi of both fine roots and soil. To examine this, studies were conducted to calculate the fungal frequency of occurrence (%) in each and across locations in fine roots and soil; and examined five abiotic factors, irrigation, geographical locations, type of plant, type of soil, and type of water to identify their effect on fungal community of fine roots and soil in terms of least squares means and standard error (LS means±SE) across twenty orchard sites. We highlight the predominant fungi in tested samples and the variation in the effects of tested abiotic factors on the fungal community of soil-borne pathogenic fungi of both fine roots and soil.

2. Materials and Methods

2.1 Samples Collection

Soil samples were collected according to [21]. Briefly, twenty orchards from different areas of Baghdad (Al-Dora, Al-Tarmiya, Zafaraniyeh and Abu Ghraib) were sampled on March 2022 (table 1). At each orchard, eight to ten soil samples (approximately 100 g of soil and fine roots) were collected from sick tree (0-30 cm depth), combined in sterile bags, and transported immediately to the laboratory of Department of Biology at Al-Iraqia University. Information of five abiotic factors, irrigation (surface and drip irrigation), location (Abu Ghraib, Tarmiyah, Dora and Al Za'franiya) (table 1), type of plant (plant species, family and common name) (table 1), type of soil (clay, loamy and sand soil), and type of water (municipal, river and well water) were collected at each orchard site.

2.2 Fungal isolation from Fine roots

Fine roots were collected according to modified method of [22]. Briefly, roots were collected from each combined soil sample using forceps. Then, they were washed with tap water to eliminate soil residue. Surface roots were sterilized using ethanol (70%) for 3min, and then washed three-times with sterile deionized-water (SDW), Subsequently, fine roots sections were plated onto Petri dishes (9cm) containing water agar medium [(WA; 2% w/v) autoclaved at 121°C/1.5 bar for 15 mins] with 25 ppm streptomycin and incubated for 2-7 days at 25°C under cool/white fluorescent light. Emerging fungal colonies were subcultured onto other Petri dishes poured with potato dextrose agar [(PDA; MilliporeSigma, USA) autoclaved at 121°C/1.5 bar for 15 mins] supplemented with 15 ppm streptomycin and maintained at 25°C under dark conditions. Hyphal tip isolation was used to provide pure isolate cultures, and these pure cultures were stored at 4°C until needed.

2.3 Fungal isolation from soil

Serial-dilution method was used to isolate fungi from soil. 1ml of each of 10^{-3} to 10^{-6} dilutions were plated onto Petri-plates (9cm) containing PDA (autoclaved at 121°C/1.5 bar for 15mins) supplemented with 25ppm streptomycin and incubated for 2-7 days at 25°C under cool/white fluorescent light. Emerging fungal colonies were subcultured onto other Petri dishes poured with PDA (autoclaved at 121°C/1.5 bar for 15mins) supplemented with 15ppm streptomycin and maintained at 25°C under dark conditions. Hyphal tip isolation was used to provide pure isolate cultures, and these pure cultures were stored at 4°C until needed.

2.4 Fungal identification

Morphological and microscopic characteristics, including colony colour, texture, along with conidial shape were examined on PDA. Isolates were grown for 5-7 days at 25°C under cool/white fluorescent light. Comparability was made with descriptions by [23-25].

2.5 Statistical analyses

Normality of data from experiment were tested using Shapiro–Wilk test before conducting analyses. One-way ANOVA analysis, using XLSTAT Excel, was preformed to calculate Least squares mean (LSM), standard error (SE) and P value of variables. Frequencies of occurrence of fungi isolated from both fine roots and soil were calculated using SPSS software program, means were analysed and compared using Tukey's test.

3. Results

3.1 Fungal community of fine roots

Orchard sites

Fungal isolates were recorded at eight orchard sites including Ab1, Ab4, T6, T8, D1- 2, and Al1-2 whereas no fungal isolates were detected at eleven orchard sites including Ab2-3, Ab5, T1-5, T7, T9-10, and D3 (table 1; Fig. 1a). The highest number of isolated fungi was in Al-Za'franiya (72.5%) followed by Dora (14.5%), Abu Ghraib (8.7%), and Tarmiyah (4.3%) (Fig. 2).

Fungal isolates

Nineteen isolates of different fungal species were isolated as followed, Ab1 (2 isolates), Ab4 (1 isolate), T6 (1 isolate), T8 (2 isolates), D1 (1 isolate), D2 (2 isolates), Al1 (1 isolate) and Al2 (9 isolates) (Fig. 1c).

Fungal identification

The morphological and microscopic characteristics of 19 isolates of 3 different fungal species, *Aspergillus niger*, *Fusarium spp.*, and *Penicillium spp.*, were comparable to the descriptions by [23,24].

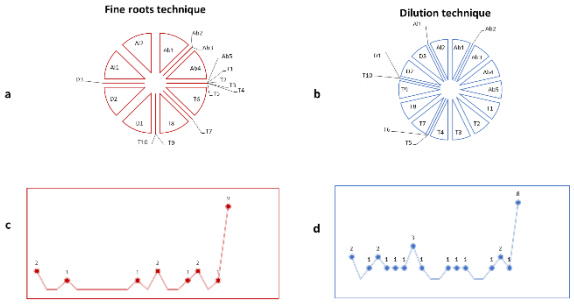

Fig. (1) Diagram of (a) fine roots method detections from 20 orchard sites, outside means no fungi isolated, while inside means fungi isolated; (b) soil dilution method detections from the same orchard sites, respectively; (c) number of isolates at each site using fine roots method; (d) number of isolates at each site using soil dilution method

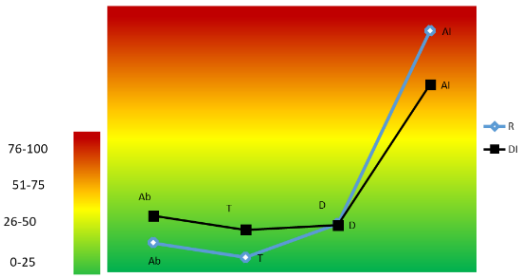

Fig. (2) Heat map of the rate of fungi isolated as by geographical locations, (Al=Al-Za'franiya, D=Dora, Ab= Abu Ghraib and T= Tarmiyah). (R, Fine roots method and D, dilution method)

Frequency of occurrence

For signal location, *Aspergillus niger* recorded (0%) in both Abu Ghraib, Tarmiyah, and Al Za'franiya, and (33.3%) in Dora. *Fusarium* spp. recorded (33.3%) in Abu Ghraib, (100%) in both Tarmiyah and Al Za'franiya, and (66.7%) in Dora. While *Penicillium* spp. recorded (66.7%) in Abu Ghraib and (0%) in both Tarmiyah, Dora and Al Za'franiya (Fig. 3).

Across four locations, *Aspergillus niger* recorded (5.3%). *Fusarium* spp. recorded (84.2%). While *Penicillium* spp. recorded (10.5%) (Fig. 4).

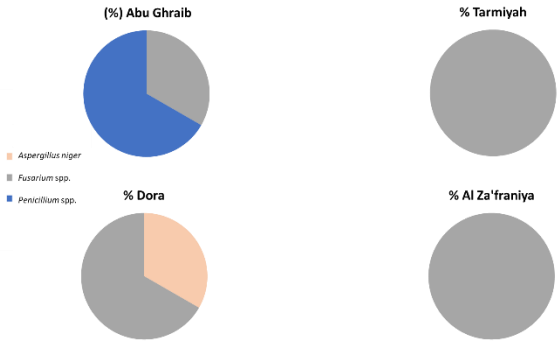

Fig. (3) Isolated fungal species in fine roots per location and their frequencies of occurrence (%)

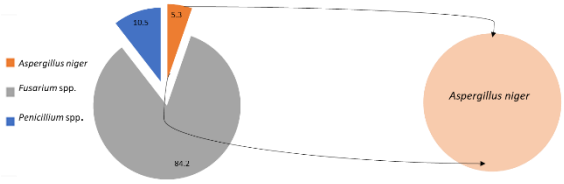

Fig. (4) Isolated fungal species in fine roots across four locations and their frequencies of occurrence (%)

Effect of abiotic factors on fungal community

For irrigation, type of soil, and type of water, there were no influence (p>0.05) on fungal community (Fig. 5a,d and e).

For location, there were a high significant (p<0.01) effect between four locations and fungal community, Al-Za'franiya revealed abundance of fungal community (5.0±1.14), followed by Dora (1.0±0.93), Abu Ghraib (0.6±0.72), and Tarmiyah (0.3±0.51) (Fig. 5b).

For type of plant, the relationships between nine plant families and fungal community showed a significant (p<0.05) effect of Lythraceae on fungal community (5.5±1.15). Whereas, others eight families had no impact on fungal community (Fig. 5c).

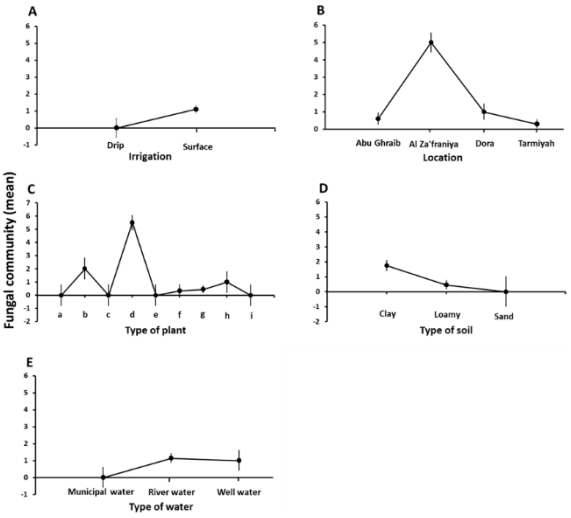

Fig. (5) Least squares means and standard error (LS means±SE) for fungal community isolated from fine roots effected by (a) Irrigation; (b) Location; (c) type of plant; (d) type of soil; and (e) type of water

3.2 Fungal community of soil

Orchard sites

Fungal isolates were recorded at fourteen orchard sites including Ab1, Ab3-5, T1-5, T7-9, D2- 3, and Al2 whereas no fungal isolates were detected at six orchard sites including T5-6, T10, D1, and Al1 (table 1; Fig. 1b). The highest number of isolated fungi was in Al-Za'franiya (56.3%) followed by Abu Ghraib (16.9%), Dora (14.1%), and Tarmiyah (12.7%) (Fig. 2).

Fungal isolates

Twenty-six isolates of different fungal species were isolated as followed, Ab1 (2 isolates), Ab3 (1 isolate), Ab4 (2 isolates), Ab5 (1 isolate), T1 (1 isolate), T2 (1 isolate), T3 (3 isolates), T4 (1 isolate), T7 (1 isolate), T8 (1 isolate), T9 (1 isolate), D2 (1 isolate), D3 (2 isolates), and Al2 (8 isolates) (Fig. 1d).

Fungal identification

The morphological and microscopic characteristics of 26 isolates of 4 different fungal species, *Aspergillus flavus*, *A. niger*, *Fusarium spp.*, and *Penicillium spp*., were comparable to the descriptions by [23-25].

Frequency of occurrence

For signal location, *Aspergillus flavus* recorded (33.3%) in both Abu Ghraib and Dora, (0%) in Tarmiyah, and (12.5%) in Al Za'franiya,. *A. niger* recorded (16.7%) in Abu Ghraib, (33.3%) in Tarmiyah, (0%) in Dora, and (62.5%) in Al Za'franiya. *Fusarium* spp. recorded (33.3%) in Abu Ghraib, (22.2%) in Tarmiyah, (66.7%) in Dora, and (25.0%) in Al Za'franiya. While *Penicillium* spp. recorded (16.7%) in Abu Ghraib, (44%) in Tarmiyah, and (0%) in both Dora and Al Za'franiya (Fig. 6).

Across four locations, *Aspergillus flavus* recorded (15.4%), *A. niger* recorded (34.6%). *Fusarium* spp. recorded (30.8%). While *Penicillium* spp. recorded (19.2%) (Fig. 7).

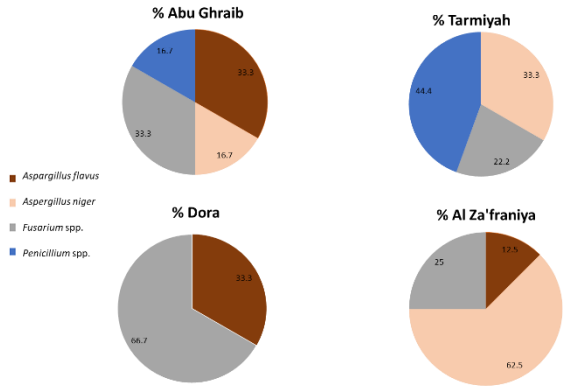

Fig. (6) Isolated fungal species in soil dilutions per location and their frequencies of occurrence (%)

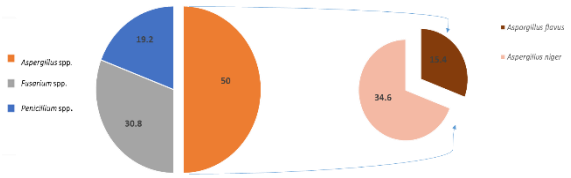

Fig. (7) Isolated fungal species in soil dilutions across four locations and their frequencies of occurrence (%)

Effect of abiotic factors on fungal community

For irrigation, type of soil, and type of water, there were no influence (p>0.05) on fungal community (Fig. 8a,d and e).

For location, the relation between four locations and distribution of fungal community showed a significant (p<0.05) effect of Al-Za'franiya location on fungal community (4.0±1.17), while there were no effects of other three locations on fungal community (Fig. 8b).

For type of plant, the relationships between nine plant families and fungal community demonstrated a significant (p<0.05) effect of Lythraceae on fungal community (5.0±1.10). Whereas, others eight families had no impact on fungal community (Fig. 8c).

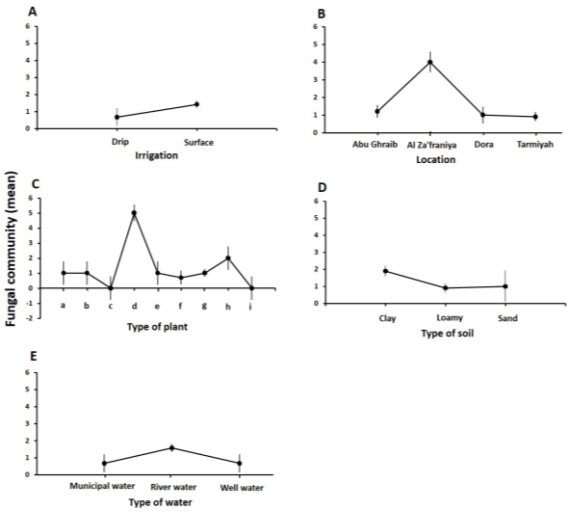

Fig. (8) Least squares means and standard error (LS means±SE) for fungal community isolated from soil dilutions effected by (a) Irrigation; (b) Location; (c) type of plant; (d) type of soil; and (e) type of water

4. Discussion

This is the first/original study to calculate the fungal frequency of occurrence (%) of fine roots and soil in each location and across four locations (20 orchard sites) in Baghdad; and to show how five abiotic factors, irrigation, geographical locations, type of plant, type of soil, and type of water, influenced the fungal community of soil-borne pathogenic fungi across twenty orchard sites. As hypothesized, fungal community varies depending upon the type of samples. Further, test abiotic factors have different influences on fungal community of soil-borne pathogenic fungi of both fine roots and soil.

Soil demonstrated the highest number of fungal isolates (26 isolates) and a high variability in fungal spp. with the predominant of *Aspargillus* spp. (≈50%) in total, followed by fine roots with 19 isolates and with the predominant of *Fusarium* spp. (>80%) in total. In general, soil is the primary reservoir of *Aspergillus* spp. despite being isolated from various environments [26]. In addition, *Aspergillus* spp. is more abundant in climates of tropical and subtropical regions within 25 to 35° latitude [27,28]. Additionally, *Aspergillus* species survived in diverse environments such as various range of temperatures, low water irrigation, and various soil hydrogen (H) ions and oxygen concentrations [29,30]. Likewise,

Fusarium spp. are considered as soil-borne fungi with facultative saprophytic characteristics on living and/or decayed organic materials [31]. The majority of tropical crops are susceptible to the invasion of *Fusarium* spp., one of the most important pathogens in agricultural cultivation [32]. Finally, variability in fungal occurrence (%) has been recorded by Haleem et al. [16] in seedling roots of forest nurseries (Forestry department and Malta) in Duhok province. In Forestry department nursery, the most predominant fungus was *Fusarium* spp. across horizontal cypress, Pine, Pistachio Robinia, and Walnut (30-54%) while *Phoma* sp. were predominant on Olive (165.67%), and *Fusarium* spp. and *Macrophomina phaseolina* (20%) on vertical cypress. In Malta nursery, the most predominant fungus was *Macrophomina phaseolina* (53% and 33%) on Olive and Pine, respectively, *Rhizoctonia solani* (56% and 39%) on Walnut and vertical cypress, respectively, *Trichoderma harzianum* (94% and 56%) on Olive and Robinia, respectively [16].

In the current study, isolated fungi, location and plant family have a significant effect on soil borne fungal community. Clearly, Geographic location is considered limiting factor on richness and abundances of bacterial and fungal community associated with fruit [33]. However, it had no influence on airborne fungal community [34]. In this study, one location affects abundance of soilborne fungal community while other three locations have no influence. This finding is consistent with that of Kasel et al. [35] who found that geographic location has a weak effect on fungal community. This also accords partly with another study that showed the high significant effect of geographic location on fungal community [33]. Another factor was plant family type, which was previously showed a positive effect on community of symbioses fungi particularly ectomycorrhizal fungi [13]. Within nine families only Lythraceae family had significantly influence on fungal community at both techniques that used in the current investigation. Lythraceae have two of the common genus in the world, *Lawsonia inermisar* and *Punica granatum*. *Lawsonia inermisar* is archaeophyte in the Middle East [36] that used as a cosmetic dye around the world [37]. *Punica granatum* is a native tree in the Middle East that is broadly cultivated [38] and has a huge industrial, medicinal, ornamental and nutritional value [39]. This plant was suffered from wilt complex disease including wilt Fusarium [40,41]. These results of previous studies could interpret the outcome in the current study which found that Fusarium spp. was the dominated genus in the roots of *Punica granatum* plants.

In parallel, three factors, soil type, irrigation and water type, showed no effects on fungal community. Soil is a “complex and dynamic environment” where the microorganisms, mostly, governed the biological activity [42]. The previous study confirmed that soil properties, including nutrient status, soil pH and soil texture have significant effect on soil fungal and bacterial community [43]. Minati & Mohammed-Ameen [20] demonstrated only three out of nine soil physio-chemical parameters, soil organic carbon and organic matter, and cation exchange capacity, showed significant differences (p<0.0000, p<0.0000, p<0.0008, respectively) in terms of disease incidence of *Fusarium* head blight and crown rot with values of 0.33-1.27%, 0.66-2.54%, and 10.08-20.59 mg/100g for the same previous parameters, respectively.

In the present study, the observed fungal community was not linked with soil type. This outcome seem to be consistent with another research which found that *Phytophthora* (organisms like fungi) community was not impacted by soil type [44]. In water type and irrigation, a little studies addressed the effect of water quality and quantity on soil fungal community in dry areas in the world [45]. Surprisingly, water at both quantity (irrigation system) and quality (water type) were not correlated with fungal community. Reasons behind no effect of three factors on fungal community, which previously recorded as main factors affect fungal community [45,46], might be that the agriculture quarantine restrictions were weak in Iraq. Indeed, the agriculture quarantine is considered a limiting factor for seed-borne and soil-borne pathogens [47]. Also, the lack of farmers awareness of biosecurity and biosafety could increase spread phytopathogens within country. In addition, cultivation mix of exotic and native trees can increase the chance to appear pathogens [48].

One interesting finding is the highest level of fungi was recorded at Al-Za'franiya and Dora locations. A possible explanation for this might be relation with human traffic. When orchards nearby human traffic, the chance of soil borne fungi spread will be increased. Another important finding is the relationship between *Aspergillus niger* and *Fusarium* species. In both fine roots and soil, the distribution of *Fusarium* spp. was influenced by *A. niger* and vice versa, when *Fusarium* spp. declined, *A. niger* increased. These outcome match those observed in earlier study [41]. *Fusarium* is a varied genus with diverse ecological features, from phytopathogens to saprotrophs and mycotoxin producers, to opportunistic pathogens of human. In this study, *Fusarium* spp. at three geographical locations was the most fungi isolated from fine roots. This result might interpret why *Fusarium* genus is the most associated fungi with plants in Baghdad.

5. Conclusion

The approach of the current study documented that *Fusarium* spp. was dominated genus in trees rhizosphere across all orchard sites of the study. This outcome could warn researchers for the risk of *Fusarium* spread in agricultural soils. The present study provided a preliminary baseline of future

studies to exam the effect of fungal community on agriculture ecosystem in Iraq. Therefore, future Investigation will be built around understanding which fungi species become invasive in agriculture ecosystem of Iraq. Further, the present study, partly, fulfilled the essential objective to find a relationship between abiotic factors and abundance of fungal community through two factors, plant families and geographical locations. However, water type, irrigation technique and soil type has no effect on fungal community.

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Table (1) the original host species used in this study, their locations, and GPS coordinates

Plant species	Plant common name	Plant family	Location	Site	Latitude	Longitude
Punica granatum	Pomegranate trees	Lythraceae	Abu Ghraib	Ab1	33.324783	43.927128
Ficus carica	Fig	Moraceae	Abu Ghraib	Ab2	33.268362	44.002766
Malus domestica	Apple	Rosaceae	Abu Ghraib	Ab3	33.239365	44.021306
Solanum melongena	Eggplant aubergine	Solanaceae	Abu Ghraib	Ab4	33.275247	44.053781
Prunus armeniaca	Cultivated apricot	Rosaceae	Abu Ghraib	Ab5	33.154041	44.220657
Ricinus communis	Castor	Euphorbiaceae	Tarmiyah	T1	33.645137	44.349952
Citrus sinensis	Sweet oranges	Rutaceae	Tarmiyah	T2	33.699995	44.411064
Citrus limon	Limon	Rutaceae	Tarmiyah	T3	33.74083	44.331413
Abelmoschus esculentus	Okra	Malvaceae	Tarmiyah	T4	33.695425	44.314933
Vigna unguiculata	Cowpea	Fabaceae	Tarmiyah	T5	33.649995	44.352699
Citrus limon	Limon	Rutaceae	Tarmiyah	T6	33.674284	44.336219
Citrus aurantium	Bitter orange	Rutaceae	Tarmiyah	T7	33.717131	44.300514
Citrus sinensis	Sweet oranges	Rutaceae	Tarmiyah	T8	33.581091	44.322486
Citrus sinensis	Sweet oranges	Rutaceae	Tarmiyah	T9	33.726269	44.402137
Citrus aurantium	Bitter orange	Rutaceae	Tarmiyah	T10	33.6493065	44.3902688
Citrus limon	Limon	Rutaceae	Dora	D1	33.223464	44.486655
Diospyros kaki	Persimmon	Ebenaceae	Dora	D2	33.191866	44.394645
Citrus reticulata	Mandarin	Rutaceae	Dora	D3	33.225761	44.482535
Eriobotrya japonica	Loquat	Rosaceae	Al Za'franiya	Al1	33.253481	44.527257
Punica granatum	Pomegranate trees	Lythraceae	Al Za'franiya	Al2	33.255204	44.526914

