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OCCURRENCE AND DISTRIBUTION OF ENTOMOPATHOGENIC FUNGI IN IRAQI AGRO-ECOSYSTEMS

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A B S T R A C T

The occurrence and distribution of entomopathogenic fungi were investigated in Iraqi province agro-ecosystems using *Galleria mellonella* bait trap technique. Over three hundred soil samples were collected from 42 sites and from specific points according to Global Positioning System (GPS) during two seasons of (2012 and 2013) and 31% of the collected samples expressed entomopathogenic fungi. Over 94 isolates were collected from the examined soil sources and sites. Results revealed that 75.5% of the isolated entomopathogenic fungi were *Beauveria bassiana* and 18.1% were *Metarhizium anisopliae* followed by *Lecanicillium lecanii* with 4.3 %. Mixed palm and citrus orchards showed the highest entomopathogenic frequency rate with 55.3% followed by only date palm orchards with 17%. South and Middle of Iraq sites revealed the highest occurrence percentage with about 90% of the isolates.

Keywords: Entomopathogenic fungi, occurrence, distribution, agro-ecosystem, GPS.

INTRODUCTION

Entomopathogenic fungi are occurred in a wide bio-diverse range of soil exemplified by forests, desert, urban regions, and orchards (Zimmermann, 1986, Lacey *et al*., 1996, Chandler *et al*., 1997, Sánchez-Peña, 1990). Soil can provide suitable environment to the entomopathogenic fungi via protecting them from UV light and other biological and non-biological factors that may limit their distribution (Keller and Zimmerman, 1989). The entomopathogenic fungi genera including *Beauveria*, *Metarhizium*, and *Paecilomyces* are usually inhabit in the soil as a part of their life cycle (Keller and Zimmerman, 1989, Domsch *et al*., 1980, Toledo *et al*., 2008), and these can also live in the soil as a saprophytic. Having the knowledge of the entomopathogenic fungi distribution and occurrence, connecting them with geographical location give an indication about the possible biological control agent that can be used to control pests (Quesada-Moraga *et al*., 2007, Shin *et al*., 2013). Imported entomopathogenic isolate may not be effective to control pests because of the environmental differences (Lockwood, 1993, Bidochka *et al*., 1998). Therefore, it is important to inspect local agroecosystem entomopathogenic fungi for incidence and biodiversity of entomopathogenic fungi to select the most active ones to apply them as biological control agents. Occurrence, distribution, and diversity of entomopathogenic fungi are affected by many different factors such as climate, soil type, cropping system, and habitats as studied in many countries such as UK, Finland, Palestine, Spain, México, Korea, Morocco, Algeria and Denmark (Shin et al., 2013, Chandler *et al*., 1997, Vänninen, 1996, Ali-Shtayeh *et al*., 2003, Quesada-Moraga et al., 2007, Keller *et al*., 2003, Zamoum *et al*., 2011, Meyling and Eilenberg, 2006, Imoulan *et al*., 2011). Previous result of Hasan *et al.* (2012) found that *B. bassiana* is the dominant entomopathogenic fungus in their study in the north of Iraq when they targeted specific sites there. However, Iraqi soils have not been investigated yet to establish exact occurrence and distribution map of entomopathogenic fungi. Therefore, the aim of the present study is to investigate the occurrence and distribution of entomopathogenic fungi in the soil of three types of orchards and agricultural agro-ecosystems of Iraq.

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MATERIAL AND METHODS

Soil sampling and preparation: Three hundred soil samples were collected over two seasons (fall and spring) of two years (2012 and 2013) from 42 sites of Iraqi agroecosystem (orchards and agricultural fields) covering the main regions of south, middle, and north (Table1, Image 1). From Five to ten soil samples from each site were collected and the distance between them ranged from 15-20m. Collected samples weight was around 500gm and taken from depth of 10-15cm after removing soil surface layer. Collected samples were incubated at 4˚C until starting isolation process (no more than one month).

Isolation of entomopathogenic fungi: Entomopathogenic fungi were isolated using bait trap method (Zimmermann, 1986) using wax moth *Galleria mellonella* L. larvae (Lepidoptera: Pyralidae) which came from the mass reared colony at 25±2˚C in the rearing room. Soil samples were sieved to remove roots and big sold materials and 40g of soil was placed into each plastic petri dish. Then, 5-6 fourth instar *G. mellonella* larvae were released in each petri dish followed by sealing them with parafilm and three replicates from each sample were used. Dishes were incubated in the dark at 25±2˚C and 50%RH in the incubator for two weeks. Checking were done every three days and dead larvae were washed with tap water and placed in a new sterile petri dish on a filter paper wetted with sterile distilled water in order to provide high level of humidity via adding water regularly; subsequently, dishes were incubated at 20˚C in the dark allowing fungal mycelia to grow over collected dead larvae. Fungal species were determined by transferring infected larvae onto two types of media according to the species: SDA for *Beauveria,* and *Lecanicillium lecanii* and PDA for *Metarhizium* and *Paecilomyces*. Then, isolates were single spored according to Scott and Chakraborty (2010), and sub-cultured many times on full PDA. The inoculum was transferred onto quarter-strength PDA plates which contain 100 µg streptomycin sulphate and 10 µg tetracycline hydrochloride mL^{-1}. Plates were incubated at 25±2˚C and 50%RH for 5 -7 days. Spore suspensions were made by adding 3-4 drops of sterile distilled water on the fungal colony that were grown on the plate using flame-sterilized loop. The spore suspensions were streaked onto 2% water agar media by using a flame-sterilized metal loop and plates were incubated under laboratory conditions for 24 h. A single germinated spore was transferred onto full-strength PDA media plate and incubated at ambient temperature. Fungal isolates were stored in 20% glycerol in 10ml tubes at three temperatures (25, 4, -20 ˚C) after giving them special cods with initials of the research institution.

Image. 1 Google map shows locations that soil samples were collected from.

Table 1. Sites of soil sources.

Province	Site	Locations	Longitude	Latitude	Soil source
Baghdad	Tarmia	3	44° 25' E	33° 64' N	Date palm
	Youssoufia	3	44° 43' E	33° 14' N	Date palm
	Kweresh	1	44° 42' E	33° 14' N	Date palm
	Khamisah	2	44° 39' E	33° 11' N	Date palm+ Citrus
	Mahmoudia	3	44° 39' E	33° 11' N	Date palm+ Citrus
	Al-Mada'in	3	44° 56' E	33° 15' N	Date palm+ Citrus
	Sakhrijah	1	44° 39' E	33° 09' N	Date palm+ Citrus
	Abu Ghraib	3	44° 30'E	33° 17 N	Trefoil fields
	Tuwaitha	1	44° 30 E	33° 12'N	Sesame fields
Wasit	Suwayrah	3	44° 49' E	33° 23' N	Date palm+ Citrus
	Aziziya	4	45° 06' E	32° 90' N	Date palm+ Citrus
	Al-Numaniyah	4	45° 24'E	32° 35' N	Date palm+ Citrus
	Al-Hafriyah	1	44° 84' E	32° 99' N	Date palm+ Citrus
	Kut	3	45° 49' E	32° 30'N	Date palm+ Citrus
	Dabuni	1	45°21' E	32°21' N	Wheat field
	Shethaif-Al Garbie	3	45°11' E	32°87' N	Date palm
	Brinaga	3	45°05' E	32°89'N	Date palm+ Citrus
	Al Zubaidiya	3	45° 40' E	32° 31'N	Date palm+ Citrus
Diyala	Diyala	2	44° 42' E	33° 63' N	Date palm+ Citrus
	Al Khalis	1	44° 56' E	33° 93' N	Date palm+ Citrus
Salah AL-din	Dujail	3	44° 27' E	33° 81' N	Grape orchards
	Balad	2	44° 43' E	34° 59' N	Grape orchards
Babil	Al-Mahawil	3	44° 39' E	32° 84' N	Date palm
Karbala	Hindiya	3	44° 19' E	32° 58' N	Date palm
	Hussainia	1	44° 06' E	32° 63' N	Date palm+ Citrus
Amarah	Amarah	4	47° 14' E	31° 86' N	Date palm
	Ali Algharbie	3	46° 69' E	31° 26' N	Date palm
	Kumayt	1	46°94' E	32°10' N	Wheat field
Dhi Qar	Nasrea-AL-jueber	4	46° 40' E	30° 59' N	Date palm
	Syd Dkhyl	3	46° 48' E	31° 13' N	Date palm
	Nasrea-AL- Mbader	2	46°44' E	31°15' N	Date palm
Al-Qadisiyyah	Al Diwaniyah	1	44° 55' E	31° 59' N	Datepalm+ Citrus
Al-Muthanna	Samawah	3	45° 17' E	31°19' N	Date palm+ Citrus
Al-Basrah	Al-Qurnah	3	47° 47' E	30° 95' N	Date palm
	Al-Haritah	2	47° 74' E	30° 67' N	Date palm
	Shatt al-Arab	4	47° 77' E	30° 44' N	Date palm+ Citrus
	AL-Hota	1	47° 78' E	30° 63' N	Date palm+ Citrus
	Abu Al-Khaseeb	5	47° 88' E	30° 47' N	Date palm+ Citrus
	Nahar Khoz	1	47° 93' E	30° 46' N	Date palm+ Citrus
Dohuk	Dohuk	5	43° 00'E	36° 52'N	Potato fields
Sulaymaniyah	Sulaymaniyah	5	45° 26'E	35° 33'N	Potato fields
Erbil	Erbil	3	44° 18' E	35° 70' N	Hills

Statistical analyses: Data were grouped in three main groups: presented entomopathogenic species, locations, and soil sources. Data were analyzed using SPSS software identifying the main significant differences in the entomopathogenic fungi frequency.

RESULTS AND DISCUSSION

The study results revealed that the entomopathogenic fungi *B. bassiana* was significantly the most frequent species (75.5%) in the soil samples followed by *M. anisopliae* with (18.1%) (Figure 1) among overall isolated entomopathogenic fungi from the all the collected samples. The fungus *Lecanicillium lecanii* scored low frequency with (4.3%) from the overall isolated fungi. *Paecilomyces lilacinus and* Fusarium *sp.* recorded the lowest frequency among other species with (1.1 %).

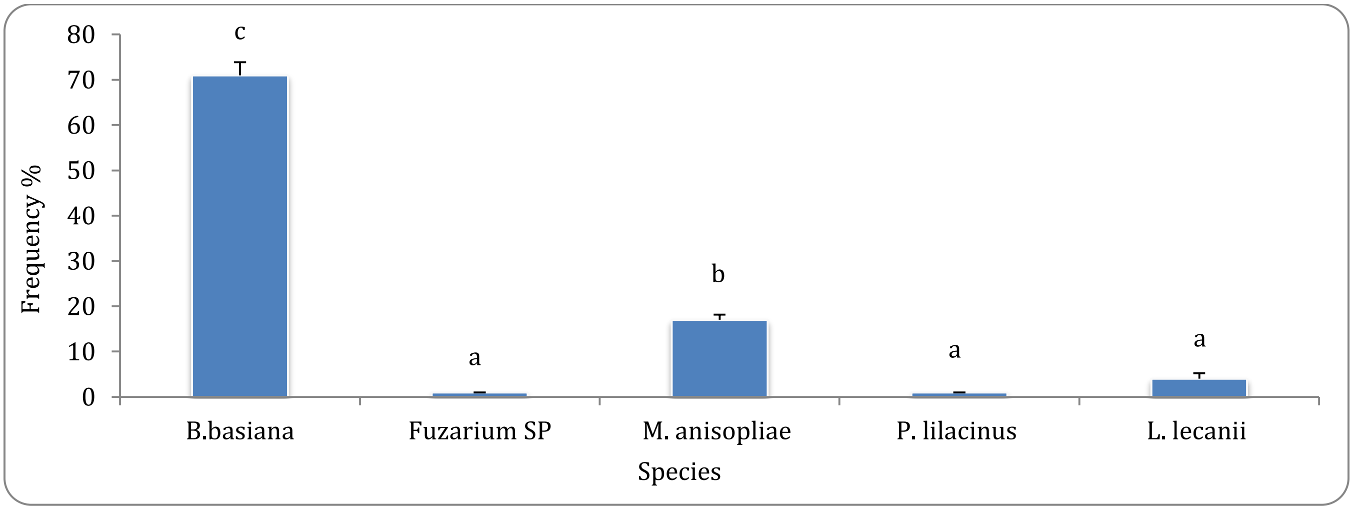

Figure 1. The frequency of entomopathogenic fungi species among the overall collected isolates and their standard error.

Figure 2 shows that Brinaga site in Wasit province recorded significantly the highest frequency of entomopathogenic fungi among the all examined sites with 19.1% followed by Al-Aziziya (Wasit) that scored 11.7% frequency of fungi and it was in Wasit as well. Al-Numaniyah (Wasit) and Abu Al-Khaseeb (Al-Basrah) revealed the same number of fungal isolates with 10.6% among the overall sites. Sites like Khamisah (Baghdad), Dujail (Salah-ad-Din), and Al-Nasrea (Di Qar) expressed moderate level of fungal frequency with 8.5, 7.4, and 6.4% respectively in comparison with other sites. All other sites revealed very low frequency rate of entomopathogenic fungi ranging from 1.1% to 3.2%.

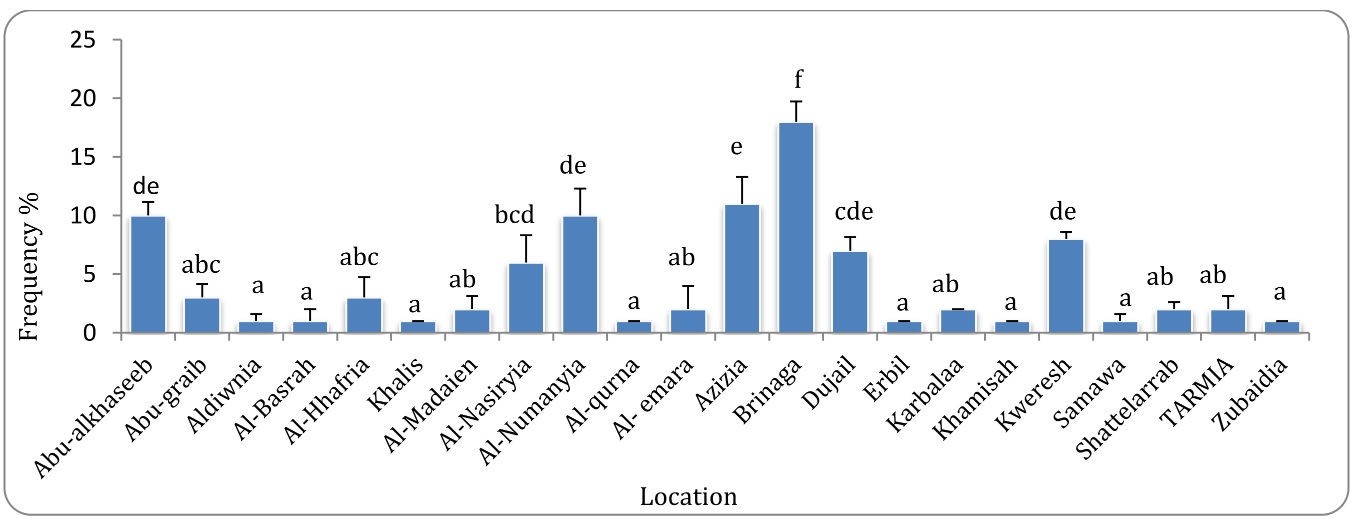

Figure 2. The frequency of entomopathogenic fungi in the examined Iraqi sites and their standard error.

The results showed (Figure 3) that the frequency of entomopathogenic fungi differ in soil samples collected from different Iraqi agroecosystems varied from orchards, crop fields, and natural topography (Figure 3). Mixed date palm and citrus orchards soil scored the highest frequency rate of entomopathogenic isolates with 55.3% among all other soil sources. Only date palm tree orchards revealed the second highest frequency proportion with 17.0% followed by only citrus tree orchards which were 10.6%. The frequency of entomopathogenic fungi in grape orchards were 9.6%. The lowest occurrence percentage of fungi which were observed in the collected samples of wheat and potato fields, plastic houses, and hills ranged from 1.1 to 4.3%.

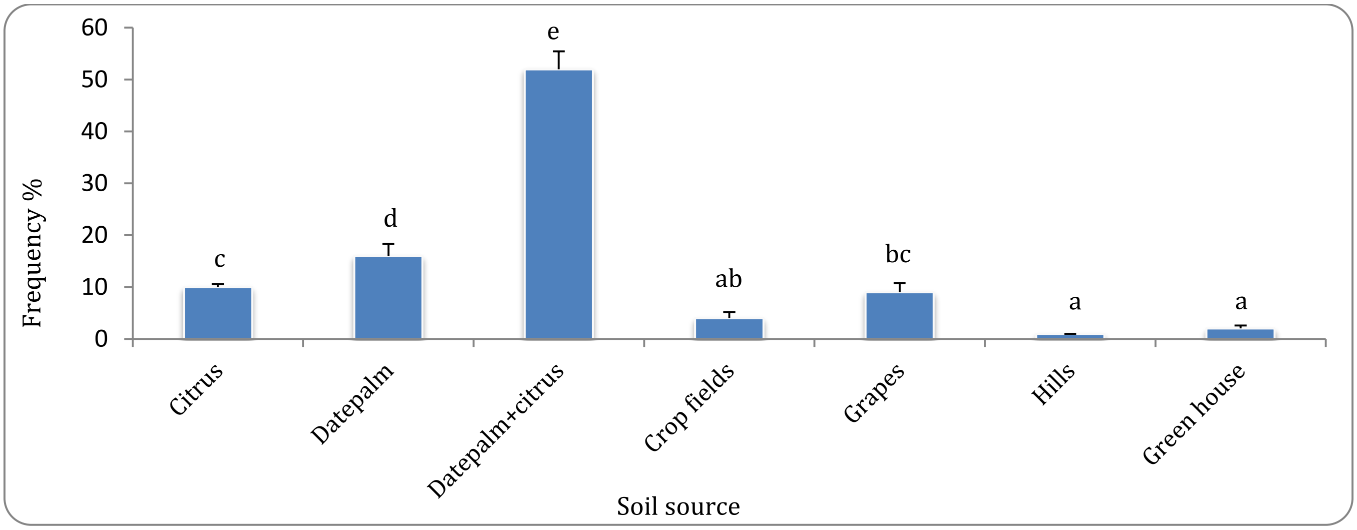

Figure 3. The frequency of entomopathogenic fungi from different Iraqi soil sources and their standard error.

The entomopathogenic fungi *B. bassiana* scored the highest frequency rate in the mixed and separate date palm and citrus orchards in comparison with other occurred fungi (Figure 4 a). Over 70% of collected isolates were *B. bassiana* in almost all the three Iraqi regions including south, middle, and north. However, over 90% of the isolates came from sites located in the middle and south of Iraq where date palm and citrus orchards are concentrated (Figure 4 b).

This confirm the previous result of Hasan *et al.* (2012) which found that *B. bassiana* is the dominant entomopathogenic fungus in their study in the north of Iraq when they targeted specific sites there. Meanwhile, Asensio *et al* (2003) found that *B. bassiana* was the most frequent entomopathogenic fungi in Alicante province in Spain with 32.8% of soils. Meyling and Eilenberg (2006) also reported that *B. bassiana* was the highest frequent in single organic agroecosystem in Denmark. Sánchez-Peña *et al* (2010) revealed the same results in Mexico using soils that were sampled from agricultural and natural ecosystem. Shin *et al.* (2013) reported that *B. bassiana* was the most frequent in Korean soils. Moreover, the current study *B. bassiana* was isolated from almost all examined agroecosystems; however, natural ecosystems showed the lowest frequency (Figure 4 a).

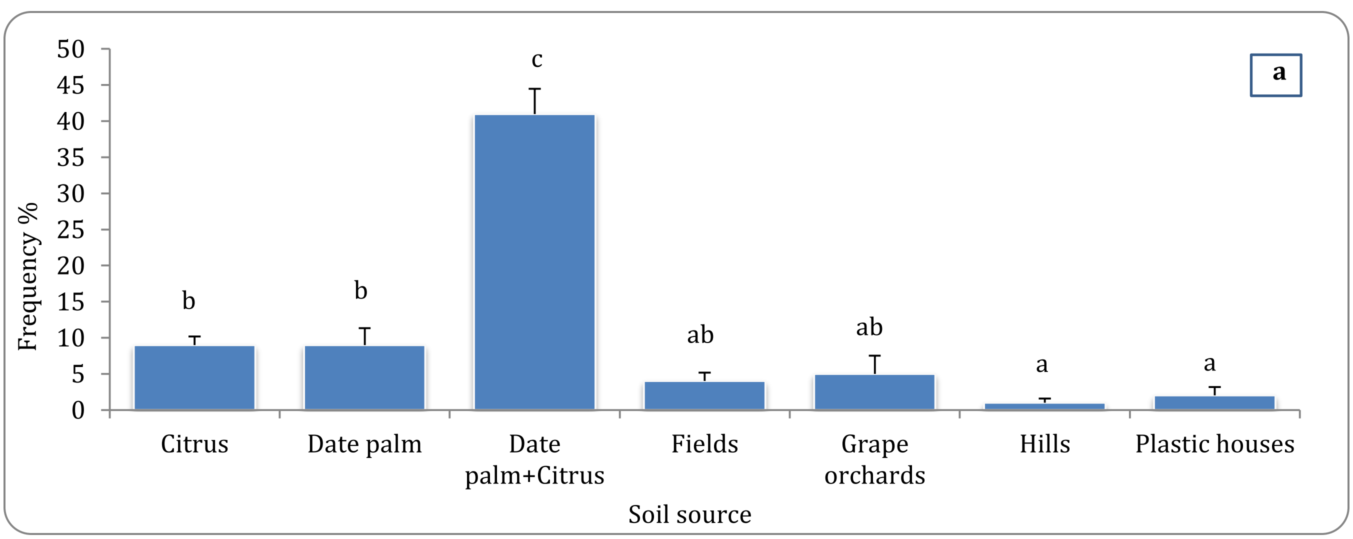

Figure 4. (a) The frequency of *B. bassiana* from different Iraqi soil sources and their standard error.

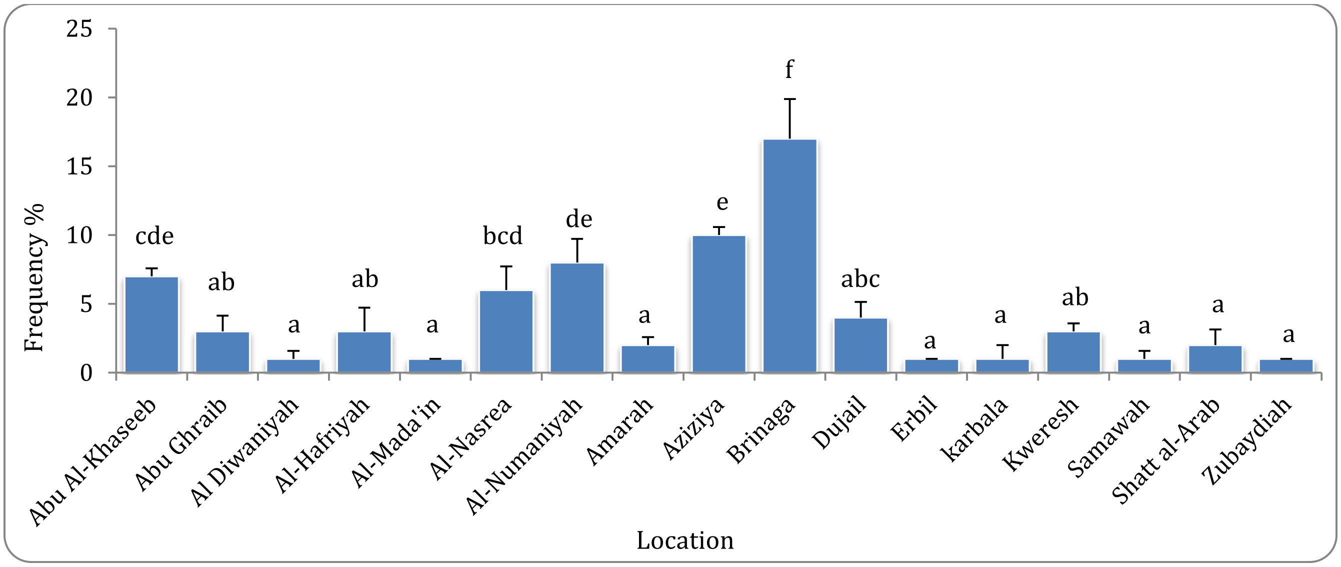

Figure 4. (b) The frequency of *B. bassiana* in the examined Iraqi sites and their standard error.

The entomopathogenic fungus *M. anisopliae* was the second highest dominant in less diverse agro-ecosystems such as citrus, date palm, date palm and citrus, and grape orchards (Figure 5 a). Moreover, their isolates were collected from limited locations with less frequency and there were no significant differences among these examined sites. Soils that expressed the highest fungal species frequency isolates might provide suitable environmental conditions, favorable food sources, and lower use of chemicals. Asensio *et al*., (2003) also found that *M. anisopliae* was the second entomopathogenic fungus in Alicante province. Most of these locations in figure (5 b) expressed *B. bassiana* as well which might mean that they are suitable for both dominant entomopathogenic species. Sánchez-Peña *et al*. (2010) as well found the same result in their study in Mexico agro- ecosystems. Shin *et al.* (2013) also mentioned that *M. anisopliae* was the second highest frequent in Korean soils. However, Meyling *et al.* (2011) reported that *M. anisopliae* was the dominant entomopathogenic fungus in Denmark; while, in China was the less frequent (Sun and Liu, 2008). This might support the hypothesis that says *M. anisopliae* occurrence required special conditions as previously mentioned.

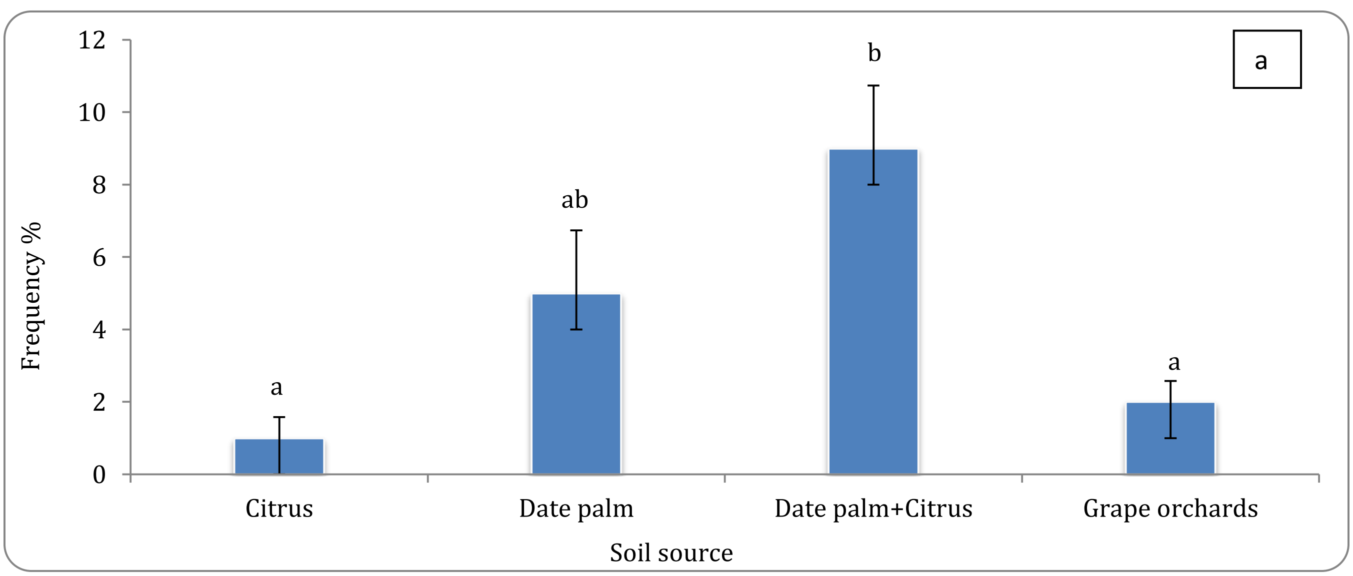

Figure 5. (a) The frequency of *M. anisopliae* from different Iraqi soil sources and their standard error.

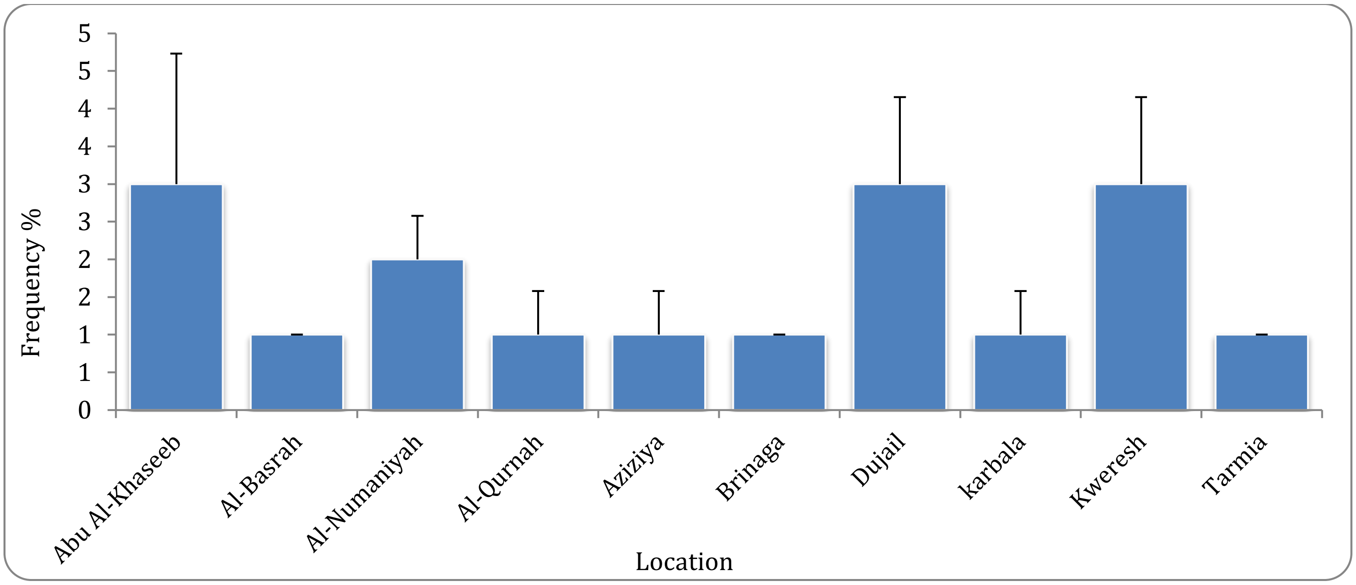

Figure 5. (b) The frequency of *M. anisopliae* in the examined Iraqi sites and their standard error.

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