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INSTITUTE OF GRADUTE STUDIES
PLANT PROTECTION DEPARTMENT

DETECTION OF *MELOIDOGYNE* SPP. FROM GREENHOUSES OF NORTHERN IRAQ

Master's Thesis

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Supervisor
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THESIS APPROVAL

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I declare that all the information given in this dissertation is true and absolute which is prepared in conformity with the regulations for Ondokuz Mayıs University Graduate School of Science and thesis writing rules. All the information was referred and kept on the right side of the laws according to scientific ethic during the stage of production of information.

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ÖZET

KUZEY IRAK SERA ALANLARINDAKİ KÖK-UR NEMATODLARININ BELİRLENMESİ

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Kök-ur nematodu türleri (*Meloidogyne* spp.), seralarda yetiştirilen sebzelerde önemli kayıplara neden olan önemli bitki paraziti nematod gruplarındandır. Kök-ur nematodları teşhislerindeki zorluklar nedeniyle Irak'ın kuzey bölgesindeki zararının, bildirilen vakalardan önemli ölçüde daha fazla olduğu tahmin edilmektedir. Bu nedenle, çalışmada Kuzey Irak'ın Süleymaniye, Erbil ve Duhok illerindeki farklı ilçelerde sebze yetiştirilen seralarda kök-ur nematodlarının yayılışı, bulaşıklık oranı ve tür teşhislerinin yapılması amaçlanmıştır. Bu bölgedeki seralarda yetiştirilen ve örnek alınan başlıca sebzeler; hıyar, domates, roka, brokoli, patlıcan, kabak, karnabahar ve maruldur. Bu sebzeler arasında ise en fazla bulaşıklığa (% 58.33) hıyar bitkisinin sahip olduğu tespit edilmiştir. İncelenen toplam 187 örnekten 70'inin (% 37,05) kök-ur nematodları ile bulaşık olduğu; bu örneklerden 35'inin Süleymaniye'de iken Erbil ve Duhok'ta ise sırasıyla 20 ve 15 olduğu saptanmıştır. En yüksek bulaşıklık oranı Süleymaniye'de (% 39.77) olup onu Duhok (% 37.50) ve Erbil'de (% 33.89) izlemiştir. Bulaşık seralardan elde edilen 70 populasyon, morfolojik (genital alan morfolojisi) ve biyokimyasal (esteraz fenotipi) yöntemler ile teşhis edilmiştir. Bulaşıklık oranının en yüksek olduğu il Sulaymaneyah (% 39.77) olup, bunu sırasıyla % 37.50 ile Duhok ve % 33.89 ile Erbil izlemektedir. Morfolojik ve biyokimyasal yöntemlerden elde edilen sonuçlar bir arada değerlendirildiğinde, en yaygın türün 45 serada saptanan *M. javanica* (% 64.3) olduğu ve onu 25 (% 35.7) seradan elde edilen *M. incognita*'nın izlediği belirlenmiştir. Çalışmada karışık populasyonun varlığına ise rastlanmamıştır. Bu çalışma, bölgedeki seralarda kök-ur nematodlarının bulaşık seviyesinin belirlendiği ve *Meloidogyne* türlerinin tanımlanması için esteraz fenotipinin kullanıldığı ilk çalışmadır.

Anahtar kelimeler: Sera, teşhis, kuzey Irak, kök-ur nematodlar.

ABSTRACT

DETECTION OF *MELOIDOGYNE* SPP. FROM GREENHOUSES OF NORTHERN IRAQ

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Root-knot nematodes are the main important group among plant-parasitic nematodes causing serious losses in greenhouses-grown vegetable over the world. Since root-knot nematodes (RKNs), *Meloidogyne* spp. are frequently misidentified, their damage is supposed to be significantly greater than the reported cases in the Northern Region of Iraq. Therefore, a survey was conducted to evaluate the distribution, infestation level, and identification of RKNs in greenhouses grown vegetables of different districts from Sulaimani, Erbil, and Duhok provinces, Iraq. RKNs were detected in 70 (37.05%) out of 187 composite soil samples, of which 35 populations were from Sulaimani followed by Erbil and Duhok with 20 and 15, respectively. The infestation level reached highest level in Sulaimani (39.77%) followed by Duhok (37.50%) and Erbil (33.89%). The major vegetable crops grown in greenhouses of this region from which samples were collected include cucumber, tomato, arugula, broccoli, eggplant, zucchini, cauliflower, and lettuce. The highest infestation level (58.33%) was detected on cucumber. Seventy populations collected from infested greenhouses, were identified based on morphological (perineal pattern) and biochemical (esterase phenotype) studies. Two species of RKNs were identified including; *M. javanica* is the most predominant species in this region detected in 45 (64.3%) greenhouses, followed by *M. incognita* which is detected in 25 (35.7%) greenhouses. The existence of mixed populations of RKNs was not detected in this study. This is the first survey study on the infestation level of root-knot nematodes in greenhouses of northern region and the first study that used esterase phenotype for identification of *Meloidogyne* spp. in Iraq.

Keywords: Greenhouses, identification, northern Iraq, root-knot nematodes.

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Hoshang Kanabi HAMAD

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SYMBOLS

%	Percent
^{o}C	Degree Celsius
M	Meter
KM	Kilometer
/	Per
Cm	Centimeter
mm	Millimeter
ml	Milliliter
µm	Micrometer
gr	Gram
mA	Milliampere
V	Volt
Cm^3	Cubic centimeter
Ha	Hectares
N	Number

ABBREVIATIONS

I1	EST phenotype of *Meloidogyne incognita*
I2	EST phenotype of *Meloidogyne incognita*
J3	EST phenotype of *Meloidogyne javanica*
PAGE	Polyacrylamide Gel Electrophoresis
RKN	Root-Knot Nematode
spp.	Species (plural)
EST	Esterase
GI	Gall index
Rm	Relative migration
M. inc.	*Meloidogyne incognita*
M. jav.	*Meloidogyne javanica*
MoWAR	Ministry of Water and Agriculture resource
MDH	Malate Dehydrogenase
TAF	Triethanolamine Formaldehyde
NaOCl	Sodium hypochlorite

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1. INTRODUCTION

Iraq is located in north temperate region between 29°15’N, and 38°15’N, 38°45 and 48°45’E with overall area of 438,320 km^2. Of these, 26 percent is suitable for agriculture and is geographically divided into three regions as the northern, central and southern regions (Jaradat, 2003). The Northern Region, with intermediate to high altitude reach altitudes of 3550 m above sea level with annual rainfall ranging between 375-724mm. The climatic condition is Mediterranean with four seasons; the winter is cold and snowy from November to April, whereas summer is dry and hot in some area and cool in another area. The region is surrounded by Turkey in north, Syria in the west and Iran in the east (Jaradat, 2003).

Greenhouse agro ecosystems, which provide proper condition for plant growth increase crop yields in a unit area. Vegetables are cultivated widely under greenhouses and open fields in northern region due to their demand and high value. Hence, it provides a suitable temperature and humidity for development of several pathogens and pests including RKNs (*Meloidogyne* spp.).

The genus *Meloidogyne* Goeldi, 1892 is the most prevalent within plant-parasitic nematodes and they distributed worldwide (Karssen et al., 2013). The parasitic life of RKNs in plants begins when the second stage juvenile that enters the root moves between the cells and reaches a suitable vascular parenchyma cell to establishing feeding site (Williamson and Gleason, 2003). Nematodes start to secrete salivary gland from dorsal esophageal glands which induce the production of giant cell. These abnormal giant cells are about 10 times larger than particular root cell, inhibit growth of the root and transmission of foods from plant roots to the nematodes (Bleve- Zacheo et al., 2007).

In the last few decades, there has been a significant development in establishing of greenhouses in Iraq due to its high yield per unit area with most of the greenhouse areas are located in the northern region. The total area is 6,414,769 m^2 (16,296 greenhouses) in northern region. Sulaimani province in the northern region is the province where greenhouse cultivation is the most intense, and the total area expressed as greenhouse is 5,610,150 m^2 (12,467 greenhouses) in 2017. Erbil is the second province where greenhouse cultivation is more intensive, and the total estimated area of cultivation under greenhouses is 919,350 m^2 (2,043 greenhouses) in this province, whereas Duhok province has 803,700 m^2 of cultivation area under greenhouse condition (Table 3.1). Cucumber (*Cucumis sativus*) and tomato (*Solanum lycopersicum*) have taken the first and the second range between vegetables grown in greenhouses of northern Iraq (Ministry of Agriculture and Water Resources, 2018).

Most of the greenhouses in this region are low-technology, that is very simple in design and climate are not controlled. There are two common types of greenhouses including tunnel greenhouse and multi-span structure greenhouse, they are usually covered by plastic and supported by steel. Most of the farmers preferred short growing season (spring and autumn) to avoid plants from cold condition. Temperature in this region fall from October to February, hence vegetables such as cucumber, tomato, eggplant (*Solanum melongena*), and peppers (*Capsicum annuum*) are grown between March to December, because they cannot withstand the cold condition.

About 100 species were described in genus of root-knot nematodes (Onkendi et al., 2014). Among them, six species are distributed widely and causes substantial damages including; *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 and there are common in warm, tropical and greenhouses of cooler areas. Meanwhile, *Meloidogyne hapla* Chitwood, 1949 (Tylenchida: Meloidogynidae), *Meloidogyne chitwoodi* Golden et al., 1980 and *Meloidogyne fallax* Karssen, 1996 are widespread in temperate climates (Karseen and Van Alest, 2001; Onkendi et al., 2014). This means that they required varied temperature for survival and reproduction of root-knot nematode species (Griffin et al.;, 1990; Karssen and Moens, 2006; Wang and Mcsorley, 2008).

Root-knot nematodes are obligate sedentary endo parasites on a huge number of plant species due to their extremely adaptive abilities (Karssen et al., 2013). Sasser (1980) reported that the most frequently distributed species of root-knot nematodes parasitize more than 2000 plant species, and they attacked most of cultivated plants. Abad et al. (2013) revealed that RKNs have more than 3,000 host plant species, this indicates an increase in the number of hosts. Root-knot nematodes cause severe damage on plants and they are considered as one of the major factors affecting both quality and quantity of yields (El-Sayed and Mahdy, 2015). Their damage is estimated to be about $100 billion/year worldwide (Bird and Kaloshian, 2003).

In the plants infested with *Meloidogyne* spp., along with the yield reduction, symptoms of the disease can appear on both aboveground and underground plant parts.

Aboveground symptom shows stunting, yellowing, reducing quality and quantity of production and recognition symptoms of nutrient deficiency due to the decrease of water and nutrient absorption (Tindall, 1983; De Waele and Elsen, 2007; Pajovic et al., 2007; Karssen et al., 2013). Underground symptoms appear as unusual enlargement of roots (galls), which is several times bigger than normal root and it has effect on absorbing and transferring water and nutrients to the plants. Rooting root may also occur during severe infection (Agrios, 2005).

They are distributed in a wide range of soil and different climatic conditions including temperate, tropical, and semitropical regions and they infect almost all plant species (Moens et al., 2009). Large number of studies on the distribution and occurrence of RKNs and their effect on vegetable crops under greenhouses and open field condition were carried out by various researchers worldwide (Sasser, 1980; Taylor et al., 1982; Netscher and Sikora , 1990; Esfahani, 2009; Sawadogo et al., 2009; Sobita et al., 2010; Aydınlı and Mennan, 2016). In Iraq, five species of *Meloidogyne* have been identified in the results of several studies which include; *M javanica*, *M incognita*, *M arenaria*, *M hapla*, and *M. cruciani* (AL_Adhami, 1955; Katcho et al., 1976; Stephan et al., 1985; Hasan et al., 2020).

Root-knot nematodes have been studied by several nematologists around the world because of their broad distribution and economically importance and they comprise main genera among plant parasitic nematodes (Trudgill and Blok, 2001; McK Bird and Kaloshian, 2003). Despite the several researches in other countries of the world, there are only few researches on the detection and identification of RKNs in Iraq. Accurate identification of *Meloidogyne* spp. is a vital and primary step for determining the most suitable measures for management of the crops (Hunt and Handoo, 2009).

Keeping view of this fact, a survey was carried out in Sulaimani, Erbil and Duhok provinces during vegetable production period in order to determine the infestation level of RKNs in the greenhouses of the region and the plant species grown in the greenhouse were also recorded and it was determined which plant species had more infestation with RKNs. The infestation level of greenhouses with root-knot nematodes were also determined according to the values of the gall scale determined on the plant roots. In addition, 70 populations of RKN which represent different locations of the region were collected and identified based on morphological (Perineal pattern) and biochemical (Esterase phenotype) methods.

2. LITERATURE REVIEWS

2.1. History of Genus

In 1855, galls were observed on greenhouse-grown cucumber by Berkeley in England which was the first sign for the emergence symptoms caused by root-knot nematodes (Hunt and Handoo, 2009). Nematodes have been explained and named differently by researchers in different states for about centuries. In 1872 Greef named them as *Heterodera radicicola*. In 1875 Licopoli named them “small worms”. Then in 1877 Jobert called them “cysts”. Root-knot nematodes *Meloidogyne* spp. were first reported by Cornu in 1879, when he found nematodes on the roots of sainfoin (*Onobrychis sativa* Lam) (Moens et al., 2009).

Then, in 1884, Carl Muller showed perineal pattern in description of root-knot nematodes for the first time. Melchior Treub (1885) extracted nematode from the roots of sugarcane and named as *Heterodera javanica* (Hunt and Handoo, 2009). Despite that two species of RKN have been named previously, but the name of *Meloidogyne* was not suggested to the genus until 1887 Göldi in Brazil for the first time suggested the name of *Meloidogyne* for root-knot nematode infected coffee and described as *Meloidogyne exigua*. Short time later, in 1889 Neal suggested the gall-forming nematode and called them *Anguillula arenaria*. During the same year, Atkinson found giant cell in the cross-section of RKN in the infected plant, although he explained as dead females instead of nutritional site. He has given a record in the history of these nematodes and referred to as *Heterodera radicola* (Jepson, 1987).

Chitwood made first revision on RKN, the genus of *Meloidogyne* in 1949, which has been proposed in 1887 by Goeldi. As he re-described three main species, *M. javanica*, *M. incognita*, and *M. arenaria*, under the genus of *Meloidogyne*. In addition, Chitwood described one new species and one new subspecies which includes *M. incognita* var. acrita and *M. hapla* (Hirschmann, 1985; Moens et al., 2009; Hunt and Handoo, 2009; Hussain, 2011). Between 1880 and 1960, only 8 species in the genus of *Meloidogyne* were described. After that the number of new species was significantly increased and 18 new species recorded in 1960s, 6 in 1970s, 30 in 1980s, 22 in the 1990s and 12 in 2000s were described (Hunt and Handoo, 2009).

RKN has changed several names in the period between early steps of describing by Berkeley in 1855 and approved *Meloidogyne* as a genus by Chitwood in 1949**.** About 100 species have been found in this genus (Onkendi et al., 2014).

The genus *Meloidogyne* has been examined very widely because of their globally wide host range and their economic importance in crop losses worldwide (Trudgill and Blok, 2001; Dong et al., 2001; Bird and Kaloshian, 2003). Sasser (1980) states that more than 95% of the species that distribute in the world consisted of *M. arenaria, M. incognita, M. javanica* and *M. hapla.* In addition, Taylor et al., (1982) collected 662 samples of RKNs from 65 distinct countries around the world. They reported that 99% of the populations consisted of four species which include *M. incognita, M. arenaria, M. hapla* and *M. javanica*. These four species of *Meloidogyne* are considered as the most widespread species of RKN in the world.

2.2. Identification of RKN Populations

Morphological Studies

Morphological diagnosis has been used for identification of RKNs for the first time by Chitwood (1949). He mentioned that the morphological features of RKN species demonstrated significant variation among species. Identification of nematode species is initially made based on the morphological characteristics of female's perineal patterns, as well as several morphological and morphometric characteristics of the second stage juvenile for several years because these are useful and fast (Khan, 2014; Almeida et al., 2008).

However, the accuracy of this method of diagnosis has been reduced due to the existence of several variations among species (Eisenback, 1985; Hirschmann, 1985; Hussey, 1985; Karseen and Van Alest, 2001). For example, misidentification between *M. inornata, M. enterolobii* and *M. incognita* is a known instance of this kind of identification due to their similarity in perineal patterns of these specimens. In addition, it's not easy to identify mixed populations only based on the morphological identification thereby other techniques of diagnosis should be use to confirm results of morphological identification (Hunt and Handoo, 2009).

2.2.1.1. Perineal pattern

Identification of species according to the perineal pattern remains as one of the main characters in the identification of *Meloidogyne* species due to their stable and common taxonomic characters (Karseen and Van Alest, 2001). Furthermore, it continues to be one of the extremely important characters used in identification of specimens (Karssen, 2002). Fingerprint-like perineal patterns located to the posterior end of female's body, which comprises from tail terminus, phasmids, lateral lines, vulva-anus region and surrounding cuticular folds or striae (Eisenback, 1985; Hirschmann, 1985). This is a low-cost technique that has been used in the identification of species, since it just needs a microscope with glycerine, lactic acid and some simple tools.

However, the technique is time consuming and requires more skills. The use of perineal patterns for diagnosis and describing of RKN species requires confirmation by using other techniques because of the high similarity between species. Using perineal patterns for the identification of RKNs has been outlined by several scientists (Taylor, 1955; Sasser and Carter, 1982; Hartman and Sasser, 1985; Eisenback, 1985; Jepson, 1987; Riggs, 1990; Charchar and Eisenback, 2000; Mitkowski, 2003).

A scanning electron microscope has been used to study the perineal pattern of *Meloidogyne* spp. (Santos, 2002). Some species of *Meloidogyne* are easily recognized due to their clear morphological characteristics, whereas others are hard to identify because of their highly perineal pattern similarity with each other. Stanley, (2002) described morphologically feature characteristics of the perineal pattern of two *Meloidogyne* spp. including *M. javanica* and *M. incognita* as follows:

Meloidogyne javanica: The perineal pattern of this species is very clear and can be easily separated from the perineal pattern of another species of *Meloidogyne*, because they have a couple of distinct lateral lines across the pattern that separates the dorsal and ventral striae (Eisenback, 1985). The striae are smooth and little wavy; dorsal arch is frequently low and rounded to high and squarish, sometimes possessing a whorl in the tail terminus area (Figure 2.1).

Meloidogyne incognita: The lateral lines in the perineal patterns of this species are absent (Figure 2.1). Perineal pattern is oval to round with a squarish high dorsal arch possessing clear whorl around the tail terminus, and striae typically smooth and wavy (Hunt and Handoo, 2009).

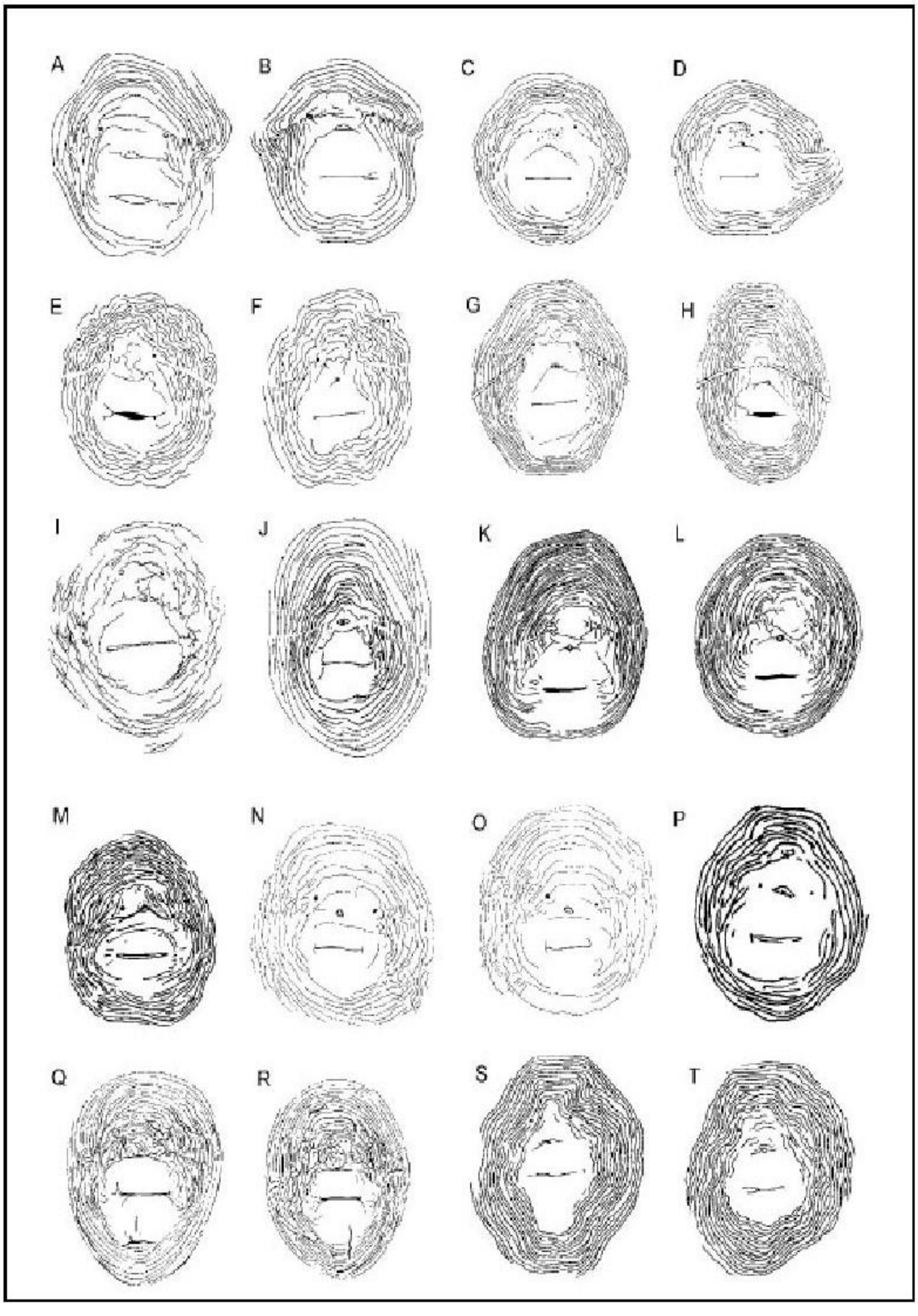


Figure 2.1. Samples of female perineal pattern used in morphological identification of RKNs. (A, B) *M. arenaria*, (C, D) *M. hapla*, (E, F) *M. incognita*, (G, H) *M. javanica*, (I) *M. acronea*, (J) *M. chitwoodi*, (K, L) *M. enterolobii*, (M) *M. ethiopica*, (N, O) *M. exigua*, (P) *M. fallax*, (Q, R) *M. graminicola*, (S, T) *M. paranaensis* (Hunt and Handoo, 2009)

Biochemical identification

The genus *Meloidogyne* has been classified primarily based on morphological and morphometric features for many years. Because of the existence of wide species and variability among perineal pattern of different species make researchers to develop another technique of identification, which is Biochemical identification. Hence, integrated traditional approaches of diagnosis with esterase phenotype and molecular identification are essential in order to get accurate and reliable identification of species. Dickson et al. (1971) noted that there have been dissimilarities among the electrophoretic patterns of several enzyme profiles, therefore several enzymes may be utilized to separate species that belong to the genus *Meloidogyne*. These enzymes include 4 dehydrogenases (lactate, malate, glucose-6-phosphate, α-glycerophosphate), 3 hydrolase (acid phosphatase, alkaline phosphatase, esterase), MDH (Malate Dehydrogenase), GDH (α-Glycerophosphate Gehydrogenase) and especially EST (Esterase).

After 1960, a large number of electrophoretic approaches were improved to identify variation of proteins. One-dimensional gel electrophoresis was the first technique that used to differentiate *Ditylenchus* from *Panagrellus* and from four *Meloidogyne* species (Myers and Benton, 1966; Dickson et al., 1971). Later, these techniques were developed further and applied in the diagnosis of RKN species.

2.2.1.2. Isozymes

Isozymes are forms of specific enzyme being different from each other regarding to their biochemical characteristics like amino acid chains and substrate regulations. Amino acid sequences have a particular change when used in electric charge and it is facilitated in the diagnosis of species. These isozymes include: esterase (EST), malate dehydrogenase (MDH), glutamate-oxaloacetate transaminase (GOT), superoxide dismutase (SOD) and glutamate dehydrogenase (GDH) (Fargette, 1987a; Carneiro et al., 2001; Cetintaş et al., 2003; Cofcewicz et al.,, 2004).

Large number of enzymatic researches indicated that the *Meloidogyne* species may be distinguished by species-specific enzyme phenotype demonstrated by polyacrylamide gel electrophoresis (PAGE). The first study on the use of enzyme phenotypes in the diagnosis of RKN was started in 1970 ((Dickson et al., 1970).

They developed a biochemically identification approach based on enzyme profiles. Esterase phenotype of 16 species from 291 *Meloidogyne* isolates have been reported, the most popular phenotypes include A2 and A3 (*M. arenaria*) I1 and I2 (*M. incognita*) and J3 (*M. javanica*) (Esbenshade and Triantaphyllou, 1985) (Figure 2.2).

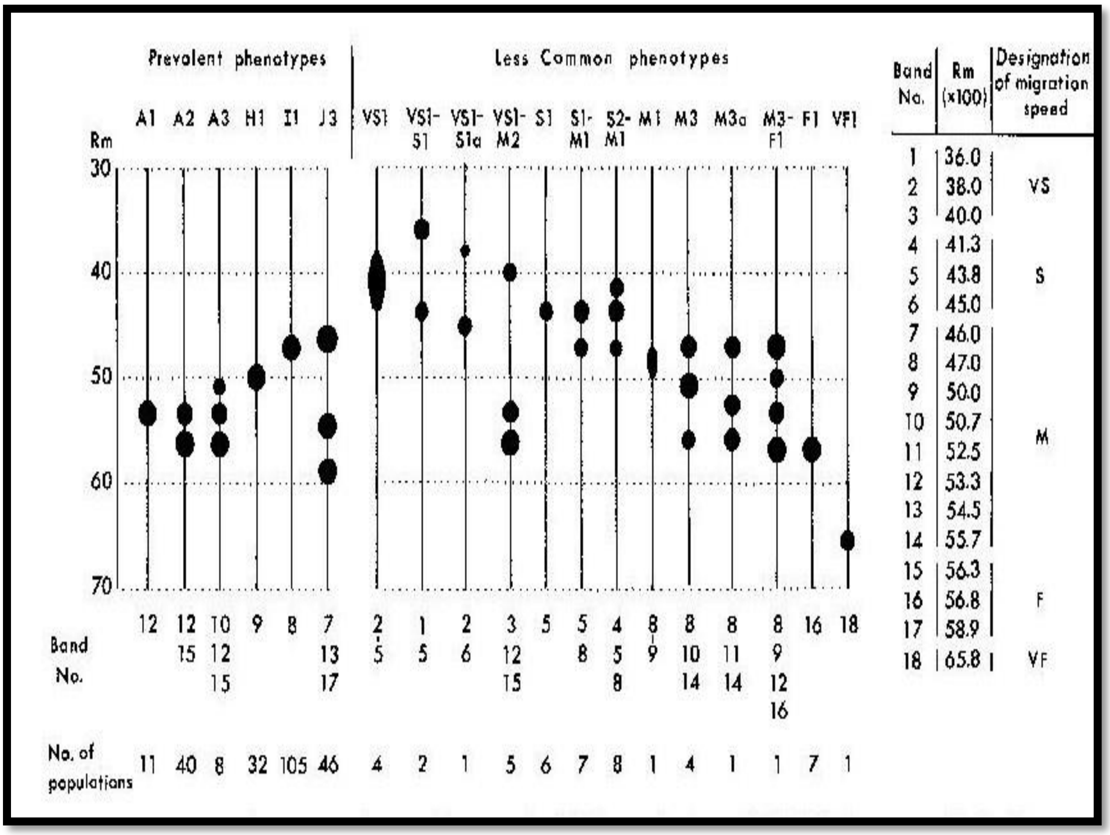


Figure 2.2. Esterase (EST) phenotype of some *Meloidogyne* species. J3 = *M. javanica*; I1, I2 = *M. incognita*; A2, A3 = *M. arenaria*; H1 = *M. hapla* (Esbenshade and Triantaphyllou, 1985)

2.2. Distribution of Root-Knot Nematodes in the World

Sasser (1980) reviewed the distribution, economic importance, and the essential knowledge for management of RKNs around the world. In this research 40 species of RKN were described and they found that only 6 species of RKNs are responsible for 95% of the damage to cultivated crops. The results of the study revealed that four species of *Meloidogyne* including *M. javanica*, *M. incognita*, *M. hapla* and *M. arenaria* are distributed widely. The samples were identified based on the morphological, host resistance, cytogenic and biochemistry techniques.

In Algeria, distribution and detection of RKN species from greenhouses of 8 different regions was studied by Sellami et al. (1999). Results of the study represented *M. javanica* as a common species in the southern regions, while *M. incognita* is dominant in the coastal regions. In addition, *M. hapla* and *M. arenaria* were also detected occasionally.

In Pakistan, several studies revealed the appearance of RKNs in various regions of the country. Different infestation level caused by root-knot nematodes ranging from 6 to 100% were recorded (Gul and Saeed, 1987; Hussain, 2011; Naz et al., 2012). Biodiversity of RKNs from Pakistan was studied by Maqbool et al., (2001). Results showed the existence of four species of *Meloidogyne* including *M. incognita*, *M. arenaria*, *M. javanica* and *M. hapla*. In addition, in this study *M. incognita* reported as a predominant specimen among RKNs in tropical zones of Pakistan, while *M. hapla* reported as prevalent species in cooler regions.

A survey was carried out to assess the incidence and identification of RKN species in Almeria and Barcelona provinces of Spain by Lucas et al., (2002). Composite soil samples of infested sites were taken from 35 greenhouses in Almeria province and from 12 greenhouses and 10 open fields in Barcelona province. Samples were diagnosed based on the morphology of female perineal pattern and esterase phenotype. Two species of *Meloidogyne* including *M. javanica* and *M. incognita* with rate 63% and 31% were identified in Almeria.

A comprehensive survey study of the RKN, *Meloidogyne* spp., associated with cowpea production areas was carried out in the 248 cowpea-grown fields in 31 states of Nigeria by Olowe, (2004). The results found *M. incognita* as a predominant species in all sampled area followed by *M. javanica* and *M. areanaria* with rate of 51.8%, 44.1%, and 4.1%, respectively.

First report of *M. enterolobii* from tomato and cucumber in northern Switzerland was published by Robertson, (2008). Samples were collected from two infested commercial greenhouses, samples were identified based on the morphological characters of female perineal pattern and confirmed by female esterase phenotype (EST) and malate dehydrogenase (MdH).

In Jordan, in an investigation three species of *Meloidogyne*, which involves *M. javanica, M. incognita* and *M. arenaria* have been reported. Furthermore, the annual yield damage due to infection by these species was estimated to be around 15% in irrigated vegetable crops. In addition, *M. javanica* reported as prevalent species in Jordan (Karajeh, 2008).

Devran and Söğüt (2009) studied identification and dissemination of RKNs in Turkey. Results showed the present of three species of RKNs, which involves *M. incognita*, *M. arenaria* and *M. javanica* with the population rate of 64.2%, 28.4% and 7.3%, respectively. Furthermore, the study indicates *M. incognita* as prevalent species of RKNs from regional greenhouses of west Mediterranean part of Turkey.

Meloidogyne javanica is a prevalent species and causes significant losses to crop plants in Iran (Moosavi, 2010). Root-knot nematodes, *M. javanica* lead to significant damage to numerous plant species like family of Cucurbitaceae (Azam et al., 2010).

In Egypt, survey and distribution of RKNs in three provinces of Egypt include Behira, Minfiya and Sharqiya was studied by (Bakr et al., 2011). In this study 54 samples were collected from field grown vegetables like tomato, eggplant, potato and pepper. Results showed that 96.26% of all surveyed fields were infested with *Meloidogyne* species. The highest population density was recorded in Behira governorate followed by Alsharqiya with 242 and 158 second-stage juveniles (j2) /250 g soil, and the lowest rate 114 j2 /250 g soil was recorded in Minfiya province.

Toumi et al., (2014) demonstrated the appearance of two species of *Meloidogyne* including *M. javanica* and *M. incognita* in greenhouse-grown tomato of Lattika and Tartus governorates in Syria.

Tuminem et al., (2015) surveyed sweet potatoes fields in Sorong district of Indonesia. A total 285 plants samples were collected and identified based on the morphological characters of female perineal pattern and PCR technique. Two species of RKN including *M. javanica* and *M. incognita* were detected in association with sweet potatoes.

Aydınlı and Mennan (2016) carried out survey for detection of root-knot nematodes from greenhouses of Middle Black Sea region of Turkey. Ninety populations of RKNs collected from greenhouses were identified based on the morphological (perineal pattern), biochemical (esterase phenotype), and molecular (PCR) approaches. The results revealed the presence of four *Meloidogyne* species including *M. arenaria*, *M. luci* (formerly *M. ethiopica*) *M. javanica* and *M. incognita*, with the presence rate of 42.2%, 41.1%, 12.2% and 4.4% respectively.

In Libya, distribution and identification of RKNs on Cucurbitaceae crops has been studied by Naeimah and Mohammed, (2017). A survey was carried out and several samples were collected in field-grown Cucurbitaceae crops in Jabal Alakther governorate. The collected samples were identified based on the morphological characters of female perineal pattern. Results of study revealed the presence of two species of *Meloidogyne*, *M. javanica* and *M. incognita* overall surveyed locations. In addition, the highest incidence reported on cucumber plants followed by zucchini and pumpkin.

2.3. Studies on Root-Knot Nematodes in Iraq

In Iraq, first report of root-knot nematode *Meloidogyne javanica* attacking 42 host plants was recorded by (AL_Adhami, 1955). A survey was carried out to detection RKNs in all provinces of Iraq. Results of the study showed that *M. javanica* was identified based on the morphological (perineal pattern) characters on 42 host plants for the first time in Iraq. This is the first detection of RKN in Iraq.

Katcho et al., (1976) reported first records of three species of *Meloidogyne* including, *M. incognita, M. arenaria,* and *M. hapla* in Iraq. A survey was conducted from several fields in northern, central and southern regions of Iraq. Several infested samples with RKNs were collected and samples were identified based on the morphological characters of perineal patterns of adult females. *M. incognita* and *M. arenaria* on watermelon, peach and broad bean were reported for the first time in all regions of Iraq. *M. hapla* was detected only in northern region of Iraq. The results also revealed the presence of mixed populations of *M. javanica*, *M. incognita* and *M. arenaria* on watermelon together. In addition, these four species of *Meloidogyne* lead to a severe damage to summer plants in Iraq.

In a survey of vineyards in the South and centre region of Iraq, 268 soil and root samples were collected during 1976 and 1978, and nematode populations were identified as *M. javanica* and *M. incognita* based on perineal pattern of adult females by Stephan et al., (1985).

Al-Saaedy and Stephan (1986) surveyed 99 eggplant fields from 17 provinces of Iraq. Nematode populations have been identified based on the perinneal pattern of adult female and differential host tests. Results indicate the detection of three species of *Meloidogyne* which include *M. javanica*, *M. incognita*, and *M. arenaria*. The most widespread species was *M. javanica* occurring in 80% of the surveyed crops. *M. incognita* was recorded as a second common occurring species of *Meloidogyne* in Iraq, whereas *M. arenaria* was detected only in few samples. Based on the results of differential host tests, *M. arenaria* race 1, *M. incognita* race 1 and race 2 were detected. This is the first study on determination of the races of *Meloidogyne* spp. in Iraq.

Stephan (1988) reported some new hosts for RKNs in Iraq. Samples of roots and soils collected from various crops in different region of Iraq. Results identified 111 plant species as host for *Meloidogyne* species. The majority of the plants were infected moderate to highly with *M. javanica* and *M. incognita* with varying population density between 120 and 10.000 J_2 / 250 g soil.

Al-Sabie and Ami (1990) conducted survey for RKN in several fields located in different districts of Duhok, Mosul and Erbil provinces. Three species of *Meloidogyne* include *M. javanica*, *M. incognita* and *M. arenaria* were identified in 28 samples. *M. javanica* is the most prevalent species and detected in 21 fields whereas *M. incognita* detected only in 1 field, *M. arenaria* in 3 fields. In addition, 3 fields were infected with mixed population of *M. javanica* and *M. incognita*.

Distribution and diagnosis of *Meloidogyne* spp. on eggplants in Ninevah province of Iraq was investigated by Ami (2006). A survey was carried out in 12 fields of eggplant in four districts of Mosul province, Iraq. The results of study showed that 93.33% of the fields infested with RKNs. Two species of *Meloidogyne* include, *M. javanica* and *M. incognita* was identified based on the morphological (perineal pattern) characters. In addition, the highest degree of infection was in a field of Salamia district (51.66%), while the lowest rate of infection detected in another field of the same district (16.66%).

Ami and Ayoub (2012) studied the susceptibility of some Cucurbitaceae plants to infection by *M. javanica*. They found that three cucumber cultivars (Ghazeer, Babylon, and Hamada) are very susceptible to *M. javanica* followed by two cultivars of watermelon (Charle and Sugar Baby) with intermediate susceptible and a cultivar of watermelon (Charleston Grey) with intermediate resistance. They also detected that a zucchini cultivar was susceptible, while two other zucchini cultivars (Hakim and Amjad) were intermediate susceptible. Among melon cultivars, one cultivar (Ananas) recorded as susceptible and the (Extra Ananas) as intermediate susceptible.

Perineal patterns of *Meloidogyne* females collected from infested roots of common zinnia (*Zennia elegans*) obtained from Erbil province were analysed by Ali et al., (2014). Additionally, North Carolina differential host test was used to identify nematode population. Based on the results, *M. javanica* were recorded for the first time on this plant species in Iraq.

Luay and Riyadh (2014) studied the incidence and identification of RKNs (*Meloidogyne* spp.) in Guba and Fadilah districts of Nineveh province, Iraq. The results of study show that 77.77% of all collected samples from eggplant were infected with one or more species of RKNs. The highest level of infestation by RKNs recorded in Guba area (54.66%). No infection by RKNs was recorded in Fadilah area. *M. javanica, M. incognia* and *M. arenaria* were detected based on the morphological (perineal pattern) identification in all collected samples. In addition, *M. javanica* was recorded as the most prevalent species in Nineveh province of Iraq on egg plant.

Incidence, diagnosis and population density of RKNs *M. javanica* on cucumber plants in Duhok province of Iraq was studied by Ami et al., (2018). A survey study was carried out in 16 greenhouse-grown cucumbers located in four different places of Semel district during both spring and autumn growing seasons. The highest incidence of root-knot nematodes (37.48%) recorded in autumn and their incidence decreases to the lowest (34.6%) in the spring. In addition, the highest infestation level (73.5%) recorded in Sartenk district and lowest (13.5%) in the Sharia district. Furthermore, *M. javanica* detected in all samples depend on the diagnosis of morphological characters of female perineal pattern.

Hasan and Abood, (2018) studied identification of RKNs according to the morphological (perineal pattern) and molecular identification in Iraq. Several root samples were collected from one field of eggplant in Babylon province, Iraq. Samples were identified based on the perineal pattern characters and molecular diagnosis. *M. javanica* and *M. incognita* were detected in samples with 73.33% and 20%, respectively. As well as 6.60% of the samples detected as mixed populations of both species.

Survey was conducted to determine the incidence, intensity and occurrence of RKNs in olive trees in Baghdad, Karbala and Babel provinces of Iraq by Waref et al., (2018). Survey was carried out in 11 fields of olive tree, 5 fields from Baghdad, 3 fields of Karbala and 1 field of Babel. The results of study revealed the presence of *M. javanica* and *M. incognita* in all surveyed locations based on morphological (perineal pattern) and molecular (PCR) techniques. The highest infestation level of 100% with average gall index 3 recorded in Al-Zafar Aniyah/ Baghdad.

Kandouh et al., (2019) tested 10 okra cultivars against *M. javanica* and *M. incognita* for host resistance. Reproduction factor (Rf) and plant shoot weight (ShW) were detected in 10 okra cultivars inoculated with 3000 egg/J_2 of *M. javanica* or *M. incognita*. Most of the tested okra cultivars were susceptible to *Meloidogyne* spp. in different levels. Lahluba cultivar was recorded as the only resistant cultivar for the both species of root-knot nematode. Reproduction factor of *M. javanica* was detected as always higher than of *M. incognita*. Whereas, ShW was extremely affected by *M. incognita* rather than *M. javanica.*

A survey was conducted by Hasan et al. (2020) and fourteen root samples were taken randomly in eggplant-grown fields in Babylon governorate of Iraq. RKNs from the samples were obtained and identified based on the morphological identification of adult female and molecular technique. This study showed that the first record of *M. cruciani* in Iraq.

Ismael and Mahmood, (2020) studied Integrated management of RKN (*Meloidogyne* spp.), collected from cucumber-grown greenhouses of Bakrajo districts in Sulaimani province, Iraq. Collected samples were identified by two methods of diagnosis including perineal pattern of adult female and PCR with species-specific primer. Two species; *M. javanica* and *M. incognita* were detected in all collected samples. The results of different controlling method revealed that use of the treatments (Nemakey, Humic acid and Chitosan) show a significant reduction in the number of nematode populations. The minimum gall index recorded on the root of cucumber plants in the case of combination in treatments.

First report of *M. javanica* on *Cestrum nocturnum* in Niynavah province, Iraq was studied by Aljuboori and Al-Hakeem, (2020). A survey was carried out and samples were collected from home garden, parks and greenhouses-grown ornamental plants to determine the host range of root-knot nematodes. Samples with the symptoms of RKN were identified depending on the morphological characters of female perineal pattern. Moreover, incidence of RKN was determined depending on gall scale 0-5. Results of morphological diagnosis reported the occurrence of *M. javanica* on night-blooming jasmine for the first time in Iraq.

Table 2.1. Table show *Meloidogyne* species found in Iraq (Al-Adhami, 1955; Katcho, 1972; Sarah et al., 2020)

Meloidogyne species	Host plants	References
Meloidogyne javanica (Treub) Chitwood, 1949	42 host plant	Al-Adhami, 1955
Meloidogyne incognita (Kofoid and White) Chitwood, 1949	120 host plant	Katcho, 1972
Meloidogyne arenaria (Neal, 1889) Chitwood, 1949	120 host plant	Katcho, 1972
Meloidogyne hapla Chitwood, 1949	water melon, peach and broad bean	Katcho, 1972
Meloidogyne cruciani Garcia-Martinez, Taylor and Smart, 1982	Eggplant	Sarah et al., 2020

3. MATERIAL METHODS

3.1. Survey of RKNs in Greenhouses of Northern Region of Iraq

Survey was conducted during November and December 2018 in order to determine the distribution of RKNs and infestation rates of vegetable grown-greenhouses of the Sulaimani, Erbil and Duhok provinces in northern region of Iraq (Figure 3.1). A total of 187 randomly selected greenhouses were surveyed for presence of *Meloidogyne* species, sampling number in each province was determined based on the number of greenhouses in the region (Table 3.1). Survey was carried out at late season of plant growth, whenever plants were minimum three months old after planting, because this period shows large number of visible galls on the plant roots. Furthermore, it helps in the recognition of infested plants with root-knot nematodes.

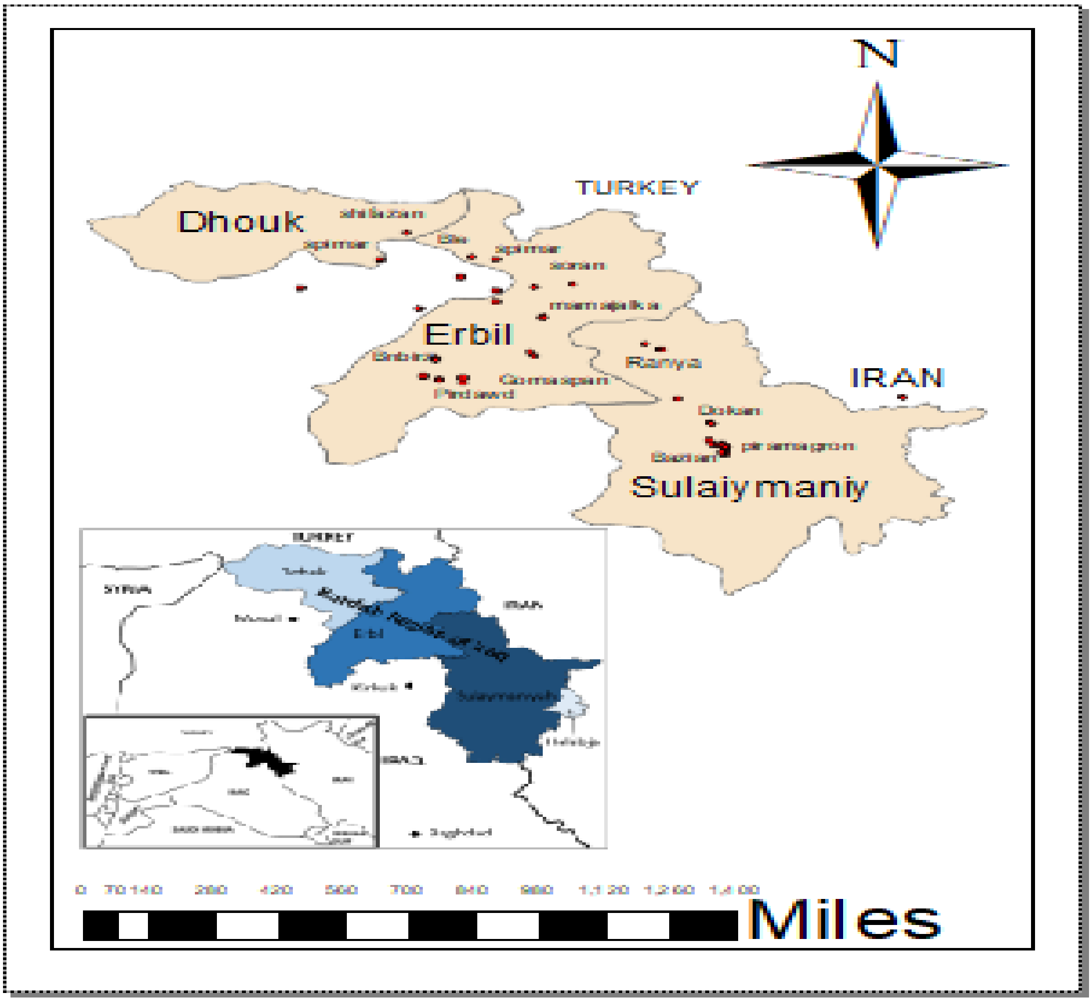


Figure 3.1. A map of north Iraq, showing three surveyed provinces for detection of the presence of *Meloidogyne* species

Sulaimani province has the largest greenhouse production in northern Iraq; Allai is the leading district where greenhouse cultivation is most intensive. Since there are large number of greenhouses in Allai district, samples were taken as coincidence from greenhouses (Figure 3.2). 88 samples have been taken from 11 districts of this province including; Allai, Qushqaya, Mahmudia, Halai, Bazian, Tasluja, Tainal, Takia, Piramagron, Dokan and Rania (Table 3.2). Erbil is the second biggest province in production of greenhouses in northern Iraq, therefore 59 samples being taken in 11 distinct places of this province including; Soran, Harir, Mamajalka, Qaryatakh, Bnberz, Mastawa, Qushtapa, Gomaspan, Pirdawd, Grdarasha and Choman. As well as 40 samples were taken from 8 districts of Duhok province include; Qasrok, Shifazan, Bjil, Chammah, Shiladz, Ble, Spimar and Bardarash. In addition, the number of examined greenhouses in each province was determined based on the greenhouse production in that province as reported by MoAWR (2017) (Table 3.1).

Table 3.1. Table show the number and area of greenhouse production in Northern Iraq*

Provinces	Number of Greenhouses	Area (m^2)	Number of examined greenhouses
Sulaimani	11.467	5.610.150	88
Erbil	3.043	919.350	59
Duhok	1.786	803.700	40
Total	16.296	6.414.769	187

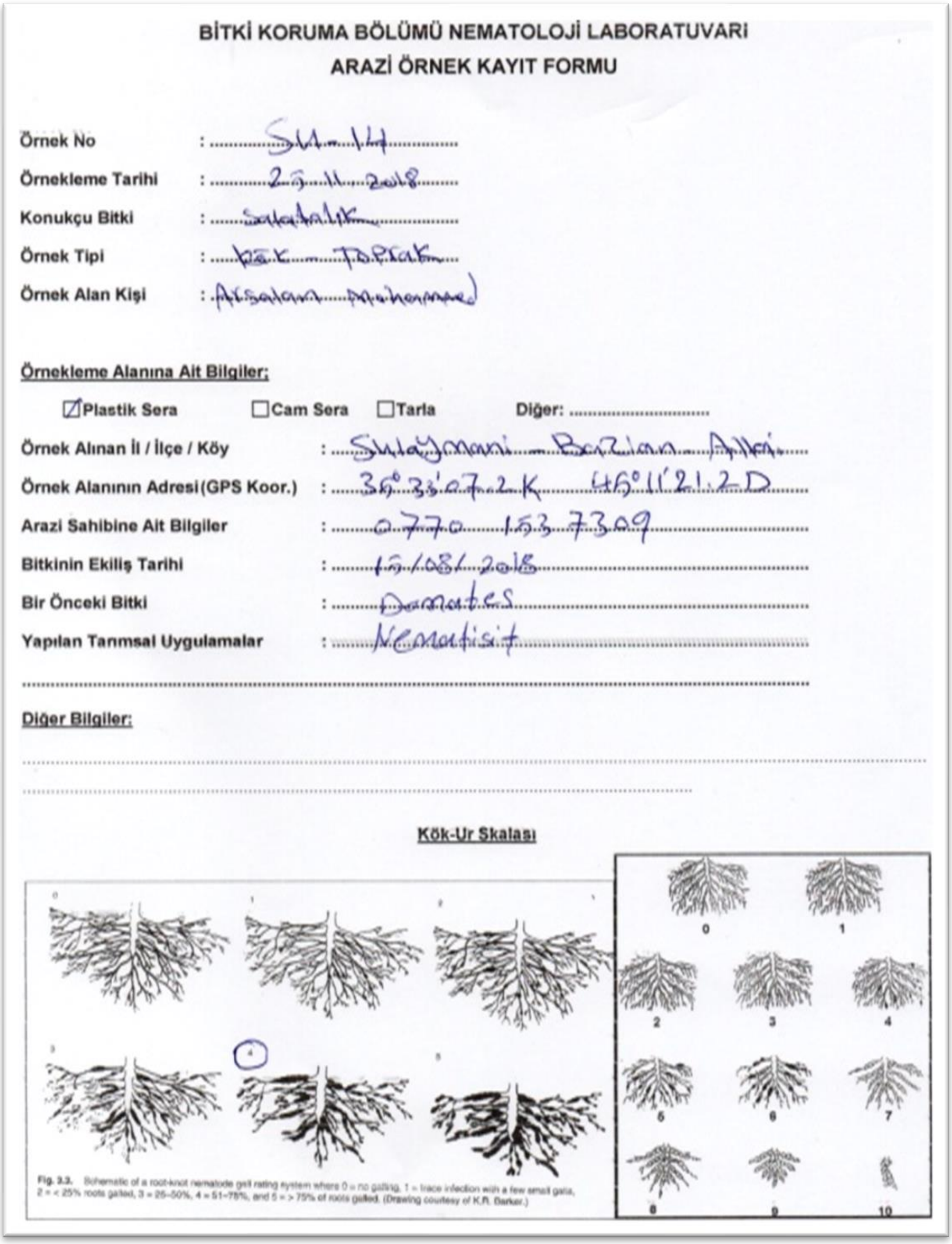
*MoAWR, 2017 (Ministry of Agriculture and Water Resources/KRG-Iraq

3.2. Collection of Root-Knot Nematode Samples

In each greenhouse 5-8 plants that showed symptoms of root-knot nematode according to the size of greenhouse were uprooted randomly as represented by Coyne et al. (2007), and the soil from rhizosphere region of infected plant (at least 1 kg) and root samples of infected plants was taken. The samples were collected by garden trowel in a depth of approximately 15-20 cm where plant roots can be definitely examined. Sub-samples were mixed to obtain composite samples for each greenhouse infected RKNs. The composite samples were put into polythene plastic bags and brought to the laboratory. The bags were tagged with sample code, location, and date was written using an indelible ink pen on adhesive paper labels fixed to the plastic bags. The garden trowel and shoes were cleaned and sterilized by using (ethanol 70%) and gloves were changed after sampling each greenhouse to avoid contamination of soil samples and disseminate of nematodes between greenhouses. As well as, the information of samples was recorded in greenhouse registration form.

Figure 3.2. Greenhouse areas from Allai district, Sulaimani province

The information of the greenhouses surveyed for RKN was recorded on "Sample Registration Form" that comprised the information on geographic coordinates, host plant type and variety, gall scale (0-5), date of planting, greenhouse age, agricultural practices and other information (Figure 3.3).



BİTKİ KORUMA BÖLÜMÜ NEMATOLOJİ LABORATUVARI
ARAZİ ÖRNEK KAYIT FORMU

Örnek No : SU-14
Örnekleme Tarihi : 25-11-2018
Konukçu Bitki : Salatalık
Örnek Tipi : Kök Toprak
Örnek Alan Kişi : Arsalan Mohammed

Örnekleme Alanına Ait Bilgiler:

☑ Plastik Sera ☐ Cam Sera ☐ Tarla Diğer: ....

Örnek Alınan İl / İlçe / Köy : Sulaymani - Bazian - Apleri
Örnek Alanının Adresi(GPS Koor.) : 35°33'07.2 K 45°11'21.2 D
Arazi Sahibine Ait Bilgiler : 0770 153 7309
Bitkinin Ekiliş Tarihi : 15/08/2018
Bir Önceki Bitki : Domates
Yapılan Tarımsal Uygulamalar : Nematisit

Diğer Bilgiler:

Kök-Ur Skalası

Figure 3.3. Sample registration form

The RKN severity in each greenhouse was determined by evaluating root galls. The soil and debris on the roots of plants were removed and washed with tap water, and then roots have been drained on the paper towel and carefully evaluated the percentage of galls on the root. The RKN severity was rated on a scale of 0 to 5, where 0 = no galling (healthy), 1 = trace infection with some minor galls, 2 = < 25% galled roots, 3 = 25-50% of galled roots, 4 = 51-75%, and 5 = > 75% of galled roots) (Barker, 1985) (Figure 3.4). The roots of all sub-samples in a greenhouse were rated for the presence of root galls and the RKN severity in each greenhouse was revealed based on the sub-sample with highest gall scale in each greenhouse. Also, the frequency of occurrence (infestation rate) of RKNs in each province was calculated based on the following equation (Carrillo-Fasio et al. 2020).

$$\text{Infestation rate: } \frac{\text{Number of greenhouses with RKN}}{\text{Total number of greenhouses surveyed}} \times 100$$

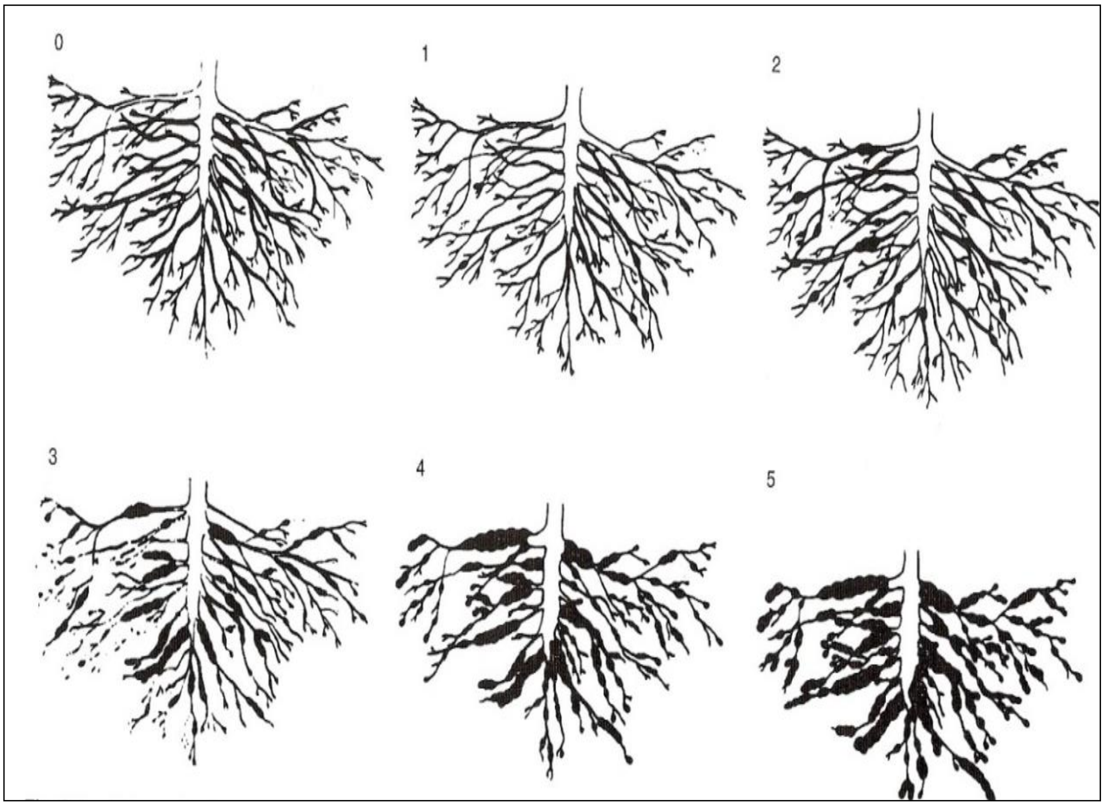


Figure 3.4. Root-knot nematode gall rating used for samples obtained from greenhouses in northern Iraq (Hussey and Janssen, 2002)

Figure 3.5. Root gall on cucumber plant caused by root-knot nematodes

Table 3.2. Sample code, location, host plants, and geographical coordinates of root-knot nematode populations that collected during survey in northern region

Sample code	province	Location	Host plant	Geographical coordinators		Altitude (M)
				Latitude (N)	Longitude (E)	
DU.1	Duhok	Qasrok	Tomato	36°37'49.17" N	44°10'38.22" E	372
DU.2		Qasrok	Cucumber	36°37'49.52" N	44°10'38.68" E	371
DU.3		Qasrok	Cucumber	36°37'47.86" N	44°10'36.75" E	373
DU.4		Chammah	Lettuce	36°37'60.45" N	44°10'17.36" E	380
DU.5		Chammah	Cucumber	36°37'58.63" N	44°10'19.52" E	372
DU.6		Shifazan	Tomato	36°38'52.23" N	43°18'33.63" E	375
DU.7		Shifazan	Tomato	36°38'50.42" N	43°18'37.93" E	404
DU.8		Shifazan	Cucumber	36°43'31.63" N	44°01'07.63" E	598
DU.9		Bjil	Cucumber	36°30'27.96" N	43°50'18.32" E	299
DU.10		Bjil	Cucumber	36°30'30.22" N	43°50'02.80" E	302
DU.11		Shiladz	Zucchini	37°01'37.51" N	43°46'43.41" E	582
DU.12		Spimar	Cucumber	36°50'30.15" N	43°39'12.02" E	586
DU.13		Spimar	Cucumber	36°50'42.85" N	43°39'42.22" E	588
DU.14		Spimar	Tomato	36°50'38.16" N	43°39'47.53" E	621
DU.15		Spimar	Tomato	36°50'39.06" N	44°10'38.68" E	632
ER.1	Erbil	Soran	Cucumber	36°39'18.08" N	44°20'54.71" E	553
ER.2		Harir	Cucumber	36°33'15.86" N	44°10'38.68" E	714
ER.3		Qaryatakh	Cucumber	36°10'22.67" N	43°54'41.15" E	362
ER.4		Qaryatakh	Cucumber	36°10'21.87" N	43°54'43.35" E	366
ER.5		Qaryatakh	Tomato	36°10'17.98" N	43°54'36.41" E	370
ER.6		Bnberz	Tomato	36°09'23.62" N	43°54'24.67" E	343
ER.7		Bnberz	Lettuce	36°09'25.82" N	43°54'34.87" E	346
ER.8		Bnberz	Lettuce	36°09'13.38" N	43°54'38.03" E	344
ER.9		Mastawa	Cucumber	36°02'29.18" N	43°51'29.56" E	311

Table 3.2. (Continue)

ER.10		Mastawa	Tomato	36°02'35.05" N	43°51'35.46" E	309
ER.11		Mastawa	Cucumber	36°02'49.68" N	43°51'19.26" E	314
ER.12		Mastawa	Cucumber	36°02'57.13" N	43°51'19.26" E	313
ER.13		Mastawa	Zucchini	36°02'57.13" N	43°51'13.76" E	310
ER.14		Qushtapa	Cucumber	36°02'11.12" N	44°01'30.17" E	394
ER.15		Qushtapa	Lettuce	36°02'09.59" N	44°02'05.38" E	402
ER.16		Qushtapa	Cucumber	36°00'14.73" N	44°01'50.94" E	394
ER.17		Qushtapa	Cucumber	36°00'09.02" N	44°01'36.14" E	390
ER.18		Pirdawd	Cucumber	36°01'13.77" N	43°55'27.50" E	340
ER.19		Pirdawd	Cucumber	36°01'14.75" N	43°55'16.18" E	339
ER.20		Pirdawd	Cucumber	36°01'18.25" N	43°55'23.93" E	338
SU.1	Sulaimani	Allai	Tomato	36°37'49.52" N	44°10'38.68" E	811
SU.2		Allai	Tomato	35°34'52.47" N	45°10'44.20" E	812
SU.3		Allai	Cucumber	35°34'53.95" N	45°10'42.32" E	812
SU.4		Allai	Cucumber	35°35'01.35" N	45°10'39.96" E	822
SU.5		Allai	Cucumber	35°34'53.40" N	45°11'02.94" E	835
SU.6		Allai	Zucchini	35°34'01.61" N	45°10'42.19" E	791
SU.7		Allai	Cucumber	35°32'51.59" N	45°11'10.06" E	774
SU.8		Allai	Cucumber	35°34'01.06" N	45°11'17.50" E	802
SU.9		Allai	Cucumber	35°34'31.91" N	45°10'04.11" E	800
SU.10		Allai	Cucumber	35°34'20.07" N	45°10'28.64" E	789
SU.11		Allai	Cucumber	35°34'10.17" N	45°10'18.34" E	780
SU.12		Mahmudia	Eggplant	35°31'13.87" N	45°11'49.13" E	862
SU.13		Mahmudia	Cucumber	35°31'16.04" N	45°11'50.74" E	841
SU.14		Mahmudia	Cucumber	35°31'21.39" N	45°11'48.69" E	831
SU.15		Qushqaya	Cucumber	35°33'09.05" N	45°11'19.17" E	808
SU.16		Qushqaya	Tomato	35°33'34.67" N	45°11'49.87" E	811

Table 3.2. (Continue)

SU.17	Qushqaya	Cucumber	35°33.14.74" N	45°11'52.08" E	789
SU.18	Qushqaya	Tomato	35°33'55.44" N	45°12'19.78" E	792
SU.19	Qushqaya	Cucumber	35°33'54.47" N	45°11'39.87" E	812
SU.20	Halai	Cucumber	35°31'17.69" N	45°10'47.99" E	866
SU.21	Halai	Arugula	35°31'04.59" N	45°10'48.45" E	857
SU.22	Halai	Eggplant	35°31'54.28" N	45°10'57.82" E	803
SU.23	Halai	Cucumber	35°31'19.70" N	45°10'46.11" E	877
SU.24	Bazian	Cucumber	35°34'08.12" N	45°08'16.45" E	785
SU.25	Bazian	Zucchini	35°34'58.22" N	45°08'36.00" E	787
SU.26	Bazian	Cauliflower	35°34'46.22" N	45°08'50.51" E	781
SU.27	Bazian	Cucumber	35°34'50.57" N	45°08'15.01" E	792
SU.28	Bazian	Tomato	35°36'27.88" N	45°07'29.14" E	823
SU.29	Piramagron	Cucumber	35°43'25.09" N	45°07'13.23" E	767
SU.30	Piramagron	Cucumber	35°43'45.49" N	45°07'43.13" E	769
SU.31	Piramagron	Arugula	35°43'23.71" N	45°08'04.44" E	771
SU.32	Piramagron	Cucumber	35°43'12.32" N	45°08'13.74" E	776
SU.33	Dokan	Cucumber	35°53'31.75" N	44°59'03.13" E	462
SU.34	Ranya	Tomato	36°13'27.58" N	44°54'47.74" E	526
SU.35	Ranya	Tomato	36°16'01.47" N	44°50'14.26" E	637

3.3. Identification of populations

3.3.1. Rearing of populations in controlled greenhouses

The positive samples for *Meloidogyne* spp. were multiplied in pots with seedling of susceptible tomato plants (*Solanum lycopersicon* L cv. Falcon). Tomato seeds were planted in the peat trays in controlled greenhouse with temperature 24 ± 2 °C until they reached 2-4 leaf seedling period. Then, seedlings transplanted into pots containing 450 cm^3 of composite soil sample collected from survey (Figure 3.6). After sixty days, samples uprooted and aerial parts of plant was cut. Females were collected under stereoscopic microscope from roots of infected plants and used in morphological (perennial pattern) and biochemical (esterase phenotype) identification

Figure 3.6. Rearing of RKN populations, *Meloidogyne* spp. in susceptible tomato cultivars (Falcon, May seed) under controlled greenhouse

3.3.2. Morphological identification

Perineal pattern, which are observed in posterior end of the female's body were used in morphological identification of *Meloidogyne* species. For this purpose, roots of infected samples that reared in greenhouses for sixty days were uprooted and washed with tap water to remove soil and debris, then galls were opened carefully by fine forceps and needle under stereoscopic microscope to pick up females. Females from each population were collected in a tube with a solution of TAF (2 ml of triethanolamine, 7 ml of formaldehyde (40%), and 91 ml distilled water) and stored until used. From each population randomly selected 8-10 females were used for preparation of perineal pattern (Figure 3.7). Females were putted into glass slide containing a droplet of 45% lactic acid. Then posterior end of female body was cut by sharp blade and inner tissues cleaned with flexible bristle under stereoscopic microscope (Figure 3.9). Cut piece from female's posterior region which containing perineal pattern were mounted in droplet of glycerine on clean glass slide (Hartman and Sasser, 1985). Later, the cover slip of slide was gently putted on it and the edges of the cover slip as closed with nail polish for permanent sealing. Finally, the samples were photographed under stereoscopic microscope and labelled by code of sample, species, and dates (3.8).

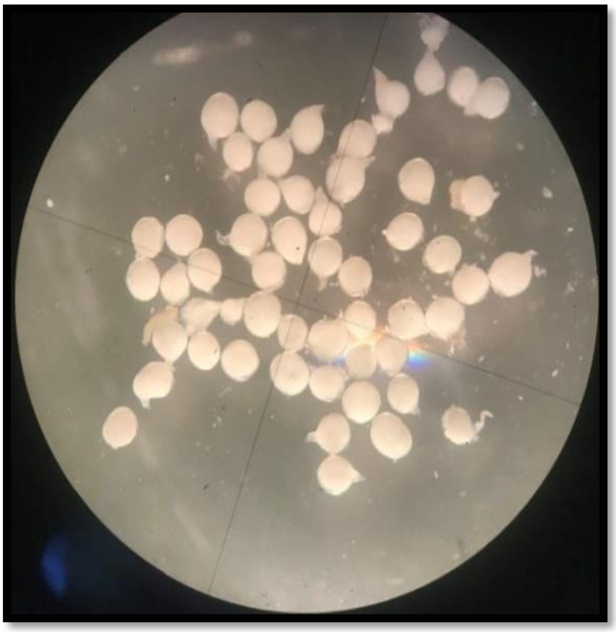


Figure 3.7. Stereomicroscope photographs of RKN females

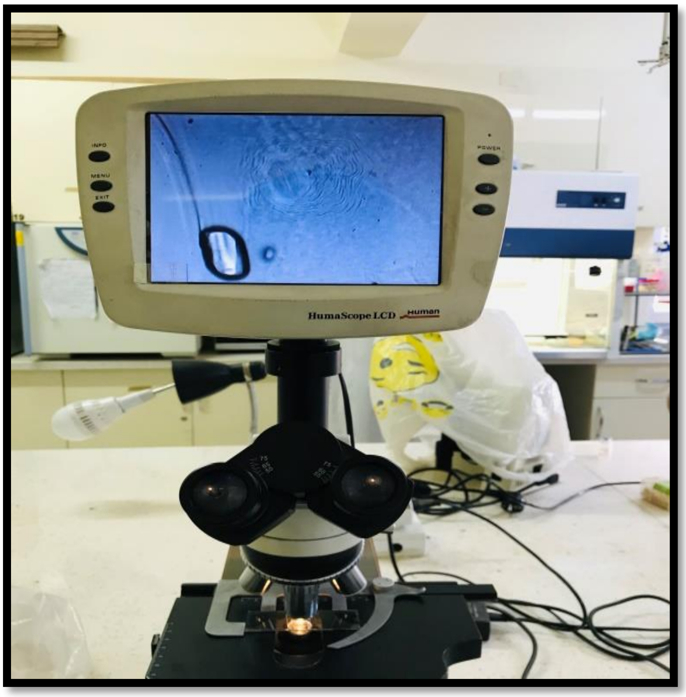


Figure 3.8. Light microscope used in detecting female perineal pattern

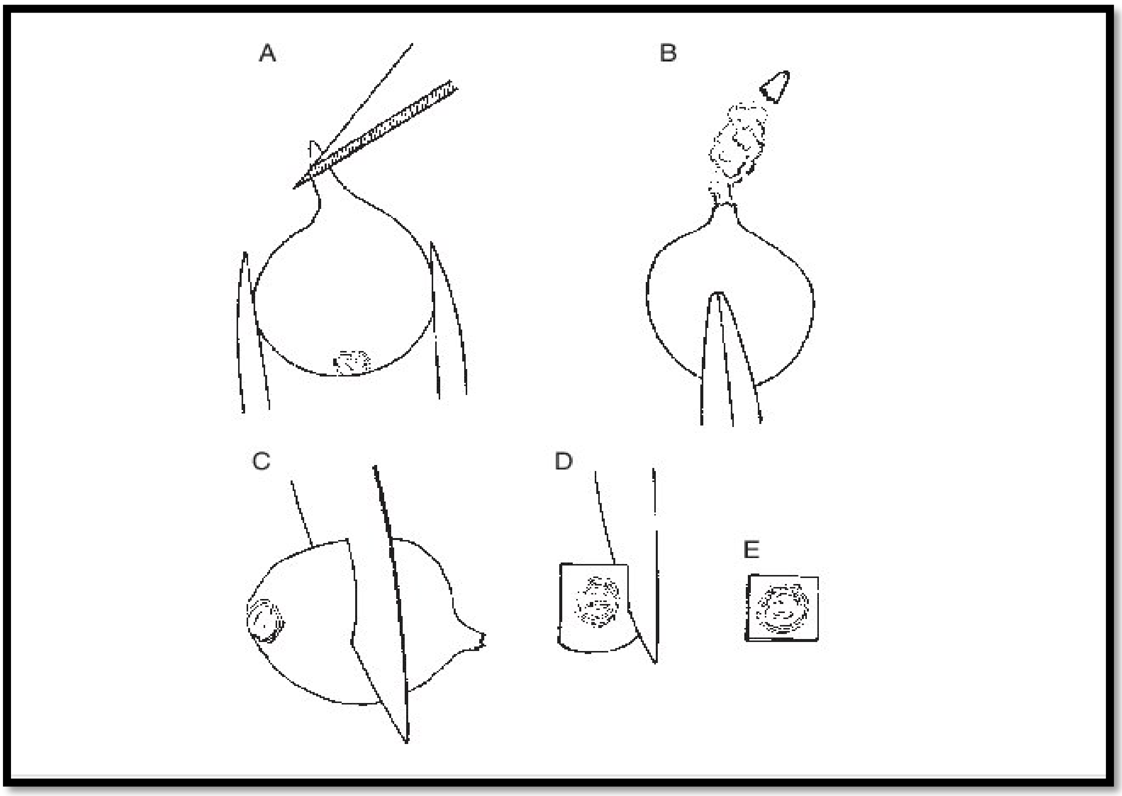


Figure 3.9. Preparation of perineal pattern from female. A, B: cutting female and removing inner tissue; C: separate posterior body with perineal pattern from rest of female body; D: Cutting cuticle around perineal pattern; E: perineal pattern ready for mounting in glycerine (Hartman and Sasser, 1985)

3.3.3. Biochemical Identification

Polyacrylamide gel electrophoresis (PAGE) was performed using Mini-Protein Tetra Cell® (Bio-Rad) for the purpose of biochemical identification of root-knot nematode populations. This part of the study consists of the following stages as described by Esbenshade and Triantaphyllou, 1985; Aydınlı and Mennan, 2016.

3.3.3.1. Preparation of extraction buffer

Micro-haematocrit glass tube was cut in half and one end of each tube was closed using a gas burner to load extraction buffer solution (20% sucrose and 1% Triton X-100). Then 5 µl from extraction buffer loaded into tubes by microliter syringe. The tubes were kept at -20 °C until used (Figure 3.10. B).

3.3.3.2. Preparation of females and standard samples

Twenty-one young female were collected from each population by opening fresh galls with fine forceps under stereoscopic microscope and then transferred to 0.9% NaCl (sodium chloride). Female individuals from each population were loaded to haematocrit glass tube (1 female/tube) containing 5 µl of extraction buffer and then crushed inside it (Figure 3.12.B). Females of *M. javanica* obtained from laboratory culture were extracted and used as standards in each gel.

Figure 3.10. (A), Preparation of micro-haematocrit glass tube by using gas burner.
(B), tubes inside refrigerator containing micro-haematocrit glass tube

3.3.3.3. Preparation of Polyacrylamide Gels

The gels were prepared two different concentrations. 7% polyacrylamide gel solution (separation gel) was firstly poured between glass plates. After gel polymerization polymerase, 3% polyacrylamide gel solution (loading gel) were poured and a comb was put to obtain the well (Figure 3.11.A). After the gel polymerization, the comb removed and glass plates were placed in the electrophoresis tank with Tris-Glycerine (8.3 pH) running buffer.

Figure 3.11. (A), Preparation of polyacrylamide gels. (B), loading of females into haematocrit tubes by using syringe

3.3.3.4. Electrophoresis

The samples at the hematocrit glass tube were centrifuged at low temperature (-5 °C) at 10,000 g for 15 minutes. Samples were loaded to wells with Bromophenol blue solution and electrophoresis tank connected to electrical power supply until the samples reached the bottom of the gels as follow (Figure 3.12. A).

First step: 18 mA / 3gel…. 15 minutes (6mA/gel)

Second step: 60 mA / 3gel…. 45 minutes (20mA/gel)

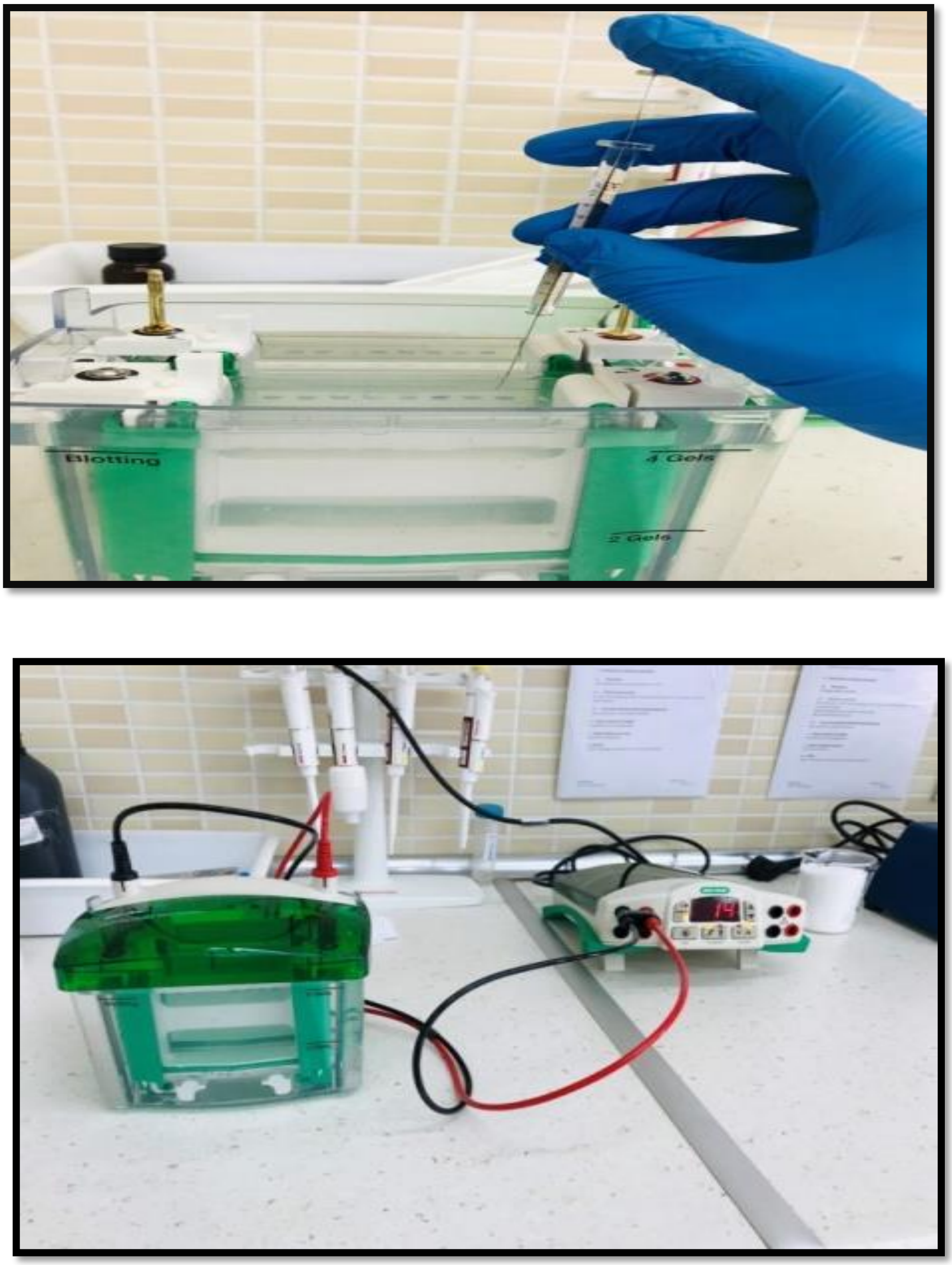


Figure 3.12. (A), loading of populations onto wells. (B), running of electrophoresis

3.3.3.5. Determination of EST Phenotype of Samples

While electrophoresis was completed, the gels gently removed from glass plates by the use of a plastic spatula. The gels were placed in a large petri dish containing dye solution and then incubated in dark condition at 37 ºC for about 30 minutes until enzyme bands became evident (Figure 3.13). The dye solutions were prepared as follow:

- Solution A prepared from:
- Sodium phosphate buffer with a concentration of 0.2M pH 7.2
- Fast blue RR

- Solution B prepared from:
- Acetone
- Alpha Naphthyl acetate

The solutions were mixed inside Erlenmeyer flask which was covered with aluminum foil and stirred until it was homogenous. Finally, the esterase phenotypes and position of bands from each population were evaluated based on the phenotype of standard sample *M. javanica* (J3). In addition, all the samples were photographed and examined by Prof. Dr. Sevilhan Mennan and Associate. Prof. Dr. Gökhan Aydınlı (Ondokuz Mayis University, Plant Protection Department, Laboratory of Nematology).

Figure 3.13. Gels in petri dish containing dye solution

4. RESULTS and DISCUSSION

4.1. Distribution and Infestation level of RKNs in Greenhouses

A total of 187 greenhouses from 30 locations in 3 provinces from north Iraq were surveyed to determine the distribution and infestation rate of RKNs. Of 187 greenhouses, 70 (37.40%) were found to be infested with RKN. The presence of RKNs has been detected in all surveyed provinces. The infestation rate of RKN were greater in Sulaimani (39.77%) than Duhok (37.50%) and Erbil (33.89%) provinces (Table 4.1). Eighty-eight greenhouses from 11 districts in Sulaimani province were surveyed for RKN. In Sulaimani, the highest number of surveyed greenhouses are located to Allai district (20 greenhouses) and RKN were found in 55% (11 greenhouses) of them indicating approximately one-third of the total greenhouses infested with RKN in this province. The number of greenhouses surveyed in other districts of this province varies from 4 to 10, while the presence of RKNs was not detected in 3 of these districts including Tasluja, Tainal, and Takia. The infestation level of greenhouses in other districts was determined to be between 25-50% as follows, Bazian (50%), Qushqaya (50%), Halai (44.44%), Piramagron (44.44%), Rania (40%), Mahmudia (37.5%), Dokan (25%) and (Table 4.2).

Table 4.1. Infestation rate of RKN and average of gall index detected in greenhouses of different provinces in northern Iraq

Provinces	Number of greenhouses surveyed	Number of greenhouses with RKN	Infestation rate (%)*	Average of gall index**
Sulaimani	88	35	39.77%	3.09
Erbil	59	20	33.89%	2.6
Duhok	40	15	37.50%	2.93
Total	187	70	37.05%	2.87

* (the number of greenhouses with RKN/number of greenhouses surveyed) x100

** (0 = no galling, 1 = trace infection with some minor galls, 2 = < 25% galled roots, 3 = 25-50% of galled roots, 4 = 51-75%, and 5 = > 75% of galled roots)

Table 4.2. Infestation rate of RKNs and number of greenhouses surveyed in Sulaimani province

Province	Location	Number of greenhouses surveyed	Number of greenhouses with RKN	Infestation rate (%)*
	Allai	20	11	55
	Qushqaya	10	5	50
	Mahmudia	8	3	37.5
	Halai	9	4	44.44
Sulaimani	Bazian	10	5	50
	Tasluja	5	0	0
	Tainal	4	0	0
	Takia	4	0	0
	Piramagron	9	4	44.44
	Dokan	4	1	25
	Rania	5	2	40
	Total	88	35	39.77

* (the number of greenhouses with RKN/number of greenhouses surveyed) x100

In Erbil province, which ranks second in terms of greenhouse area after Sulaimani in the Northern Region, RKN survey was carried out in 59 greenhouses from 11 different districts. The number of greenhouses surveyed in the districts of this province varies from 3 to 8, while the presence of RKNs was not detected in 4 of these districts including Choman, Gomaspan, Mamajalka and Grdarasha. In Mastawa area, which is most cultivation greenhouse area in Erbil, 5 (62.50%) out of 8 greenhouses were found infested with RKNs. The infestation level of greenhouses in other districts were determined as follows, Pirdawd (60%), Qushtapa (57.12%), Bnberz (50%), Qaryatakh (37.5%), Harir (33.33%) and Soran (20%) (Table 4.3).

Table 4.3. Location and infestation level of greenhouses from Erbil province

Province	Location	Number of greenhouses surveyed	Number of greenhouses with RKN	Infestation rate (%)*
	Soran	5	1	20.00
	Harir	3	1	33.33
	Mamajalka	4	0	0.00
	Qaryatakh	8	3	37.5
Erbil	Bnberz	6	3	50.00
	Mastawa	8	5	62.50
	Qushtapa	7	4	57.12
	Gomaspan	5	0	0.00
	Pirdawd	5	3	60.00
	Grdarasha	4	0	0.00
	Choman	4	0	0.00
Total	59	20	33.89	Total

* (the number of greenhouses with RKN/number of greenhouses surveyed) x100

In Duhok province, survey conducted 8 different districts, it was determined that 15 (37.5%) of the 40 greenhouses surveyed were infested with RKNs. It was noted that 5 districts were infested with RKNs as fallow: Spimar (57.12%), Qasrok (50%), Shifazan (42.85%), Bjil (40%), and Shiladz (20%). In addition, infestation with RKN was not detected in Chammah, Ble, and Bardarash ditricts (Table 4.4).

Table 4.4. Location and infestation level of greenhouses from Duhok province

Province	Location	Number of greenhouses surveyed	Number of greenhouses with RKN	Infestation rate (%)*
	Qasrok	6	3	50.00
	Shifazan	7	3	42.85
	Bjil	5	2	40.00
Duhok	Chammah	3	0	0.00
	Shiladz	5	1	20.00
	Ble	3	0	0.00
	Spimar	7	4	57.14
	Bardarash	4	0	0.00
	Total	40	15	37.50

* (the number of greenhouses with RKN/number of greenhouses surveyed) x100

Plant roots were evaluated according to a scale of 0-5 in order to determine the RKN severity in the greenhouses of this region. According to this evaluation, the greenhouse ratio in which the RKN infestation is not detected in the plant roots including, Sulaimani 60.22% (53 greenhouses), Erbil 66.10% (39 greenhouses) and Duhok 62.50% (25 greenhouses), whereas, results show that GI.1 observed in 17 greenhouses, GI.2 in 13, GI.3 in 14, GI.4 in 11 and GI.5 observed in 15 greenhouses (Table 4.8). The average gall index from Sulaimani (3.08) followed by Duhok and Erbil with (2.93 and 2.6) respectively (Table 4.4). The highest scale value 5 was detected in 15 (21.42%) greenhouses, 9 from Sulaimani, 3 from Erbil and 3 from Duhok (Table 4.7).

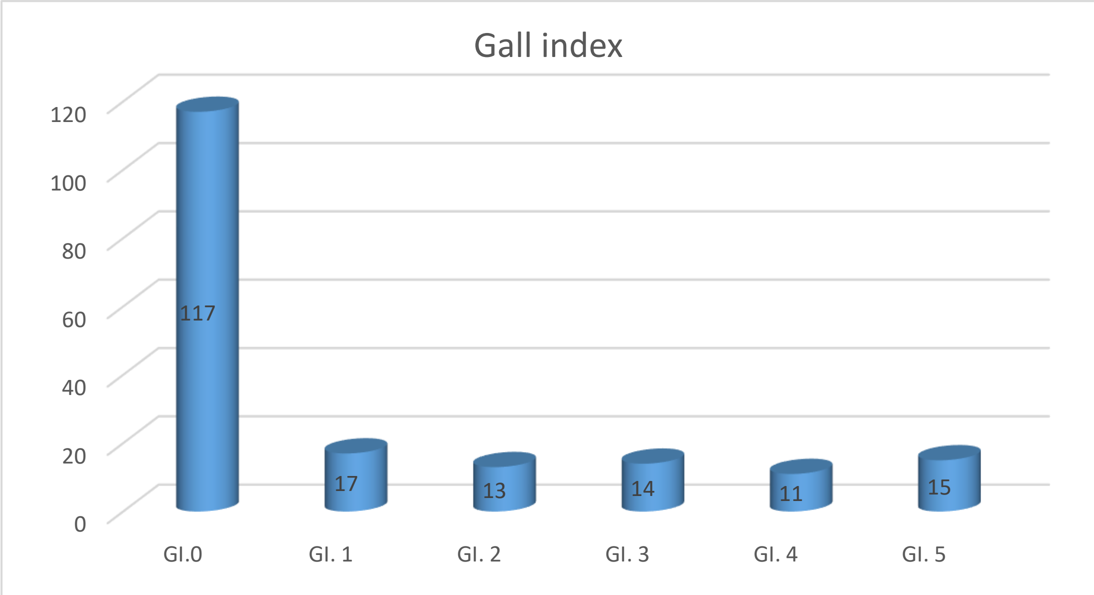


Figure 4.1. The number of root gall index value detected in all greenhouses surveyed in Northern Iraq. (0 = no galling, 1 = trace infection with some minor galls, 2 = < 25% galled roots, 3 = 25-50% of galled roots, 4 = 51-75%, and 5 = > 75% of galled roots)

The major vegetable crops grown in greenhouses of this region from which samples were collected include cucumber, tomato, arugula (*Eruca sativa*), broccoli (*Brassica oleracea* var. italica), eggplant, zucchini, cauliflower (*Brassica oleracea* var. botrytis), and lettuce (*Lactuca sativa*).

Approximately 39% of the greenhouses in northern region were cultivated with cucumber, 42 (58.33%) out of 72 cucumber-grown greenhouses were infested with RKNs. It was determined that 24% of greenhouses tomato cultivation was carried out, of which 15 (33%) out of 45 greenhouses were infested with RKNs, 2 (25%) out of 8 eggplant-grown greenhouses, 4 (21%) out of 19 zucchini-grown greenhouses, 2 (20%) out of 10 arugula-grown greenhouses, 4 (20%) out of 20 lettuce-grown greenhouses, 1 (14.28%) out of 7 cauliflower-grown greenhouses, and infestation of RKNs were not detected in broccoli-grown greenhouses. The infestation level of the vegetables grown broccoli-grown greenhouses. The infestation of RKN in the vegetables grown in greenhouses surveyed in the provinces and districts is given in Table 4.6.

Table 4.5. The infestation rate of RKN in host plants cultivated in greenhouses of Sulaimani, Erbil and Duhok provinces

Hosts	Sulaimani			Erbil			Duhok		
	Number of greenhouses surveyed	Number of greenhouses with RKN	Infestation rate (%)	Number of greenhouses surveyed	Number of greenhouses with RKN	Infestation rate (%)	Number of greenhouses surveyed	Number of greenhouses with RKN	Infestation rate (%)
Arugula (*Eruca sativa*)	3	0	0	6	2	33.33	1	0	0
Broccoli (*Brassica oleracea* var. italica)	2	0	0	2	0	0	2	0	0
Cauliflower (*Brassica oleracea* var. botrytis)	4	1	25	2	0	0	1	0	0
Cucumber (*Cucumis sativus*)	34	20	58.82	22	12	54.54	16	10	62.5
Egg plant (*Solanum melongena*)	6	2	33.33	1	0	0	1	0	0
Lettuce (*Lactuca sativa*)	10	2	20	8	2	25	2	0	0
Tomato (*Solanum lycopersicum*)	22	8	36.36	12	3	25	11	4	44
Zucchini (*Cucurbita pepo*)	7	2	28.57	6	1	16.66	6	1	16.66
Total	88	35	39.77	59	20	33.89	40	15	37.5

Table 4.6. The infestation rate of RKN in host plants cultivated in greenhouses of northern Iraq

Host	Number of greenhouses surveyed	Number of greenhouses with RKN	Infestation rate (%)*
Arugula	10	2	20
Broccoli	6	0	0
Cauliflower	7	1	14.28
Cucumber	72	42	58.33
Eggplant	8	2	25
Lettuce	20	4	20
Tomato	45	15	33.33
Zucchini	19	4	21
Total	187	70	37.43%

Table 4.7. The rate of gall scale values determined in the surveyed greenhouses of northern Iraq

Gall scale*	Sulaimani		Erbil		Duhok	
	Number of greenhouses surveyed	Rate (%)	Number of greenhouses surveyed	Rate (%)	Number of greenhouses surveyed	Rate (%)
0	53	60.22	39	66.10	25	62.5
1	9	10.22	5	8.47	3	7.5
2	4	4.54	5	8.47	4	10
3	6	6.81	6	10.16	2	5
4	7	7.95	1	1.69	3	7.5
5	9	10.22	3	5.08	3	7.5

*Root Gall Scale (0-5); 0 = no galling, 1 = trace infection with some minor galls, 2= < 25% galled roots, 3 = 25-50% of galled roots, 4 = 51-75%, and 5 = > 75% of galled roots (Barker, 1985).

Table 4.8. Gall scale values and host plant of root-knot nematode (*Meloidogyne* spp.) populations obtained from the greenhouses of Sulaimani, Erbil, and Duhok provinces in the Northern Iraq

Green house code*	Location	Host plant	Gall scale (0-5) **	Green house code*	Location	Host plant	Gall scale (0-5) **
DU.1	Qasrok	Tomato	5	SU.1	Allai	Tomato	3
DU.2	Qasrok	Cucumber	2	SU.2	Allai	Tomato	4
DU.3	Qasrok	Cucumber	1	SU.3	Allai	Cucumber	5
DU.4	Chammah	Lettuce	1	SU.4	Allai	Cucumber	3
DU.5	Chammah	Cucumber	3	SU.5	Allai	Cucumber	4
DU.6	Shifazan	Tomato	5	SU.6	Allai	Zucchini	4
DU.7	Shifazan	Tomato	4	SU.7	Allai	Cucumber	2
DU.8	Shifazan	Cucumber	3	SU.8	Allai	Cucumber	1
DU.9	Bjil	Cucumber	4	SU.9	Allai	Cucumber	2
DU.10	Bjil	Cucumber	4	SU.10	Allai	Cucumber	5
DU.11	Shiladz	Zucchini	2	SU.11	Allai	Cucumber	4
DU.12	Spimar	Cucumber	2	SU.12	Mahmudia	Eggplant	2
DU.13	Spimar	Cucumber	1	SU.13	Mahmudia	Cucumber	3
DU.14	Spimar	Tomato	2	SU.14	Mahmudia	Cucumber	4
DU.15	Spimar	Tomato	5	SU.15	Qushqaya	Cucumber	5
ER.1	Soran	Cucumber	1	SU.16	Qushqaya	Tomato	4
ER.2	Harir	Cucumber	2	SU.17	Qushqaya	Cucumber	1
ER.3	Qaryatakh	Cucumber	1	SU.18	Qushqaya	Tomato	2
ER.4	Qaryatakh	Cucumber	5	SU.19	Qushqaya	Cucumber	1
ER.5	Qaryatakh	Tomato	1	SU.20	Halai	Cucumber	3
ER.6	Bnberz	Tomato	3	SU.21	Halai	Arugula	1
ER.7	Bnberz	Lettuce	2	SU.22	Halai	Eggplant	5
ER.8	Bnberz	Lettuce	3	SU.23	Halai	Cucumber	1

Table 4.8. (Continue)

ER.9	Mastawa	Cucumber	3	SU.24	Bazian	Cucumber	4
ER.10	Mastawa	Tomato	3	SU.25	Bazian	Zucchini	5
ER.11	Mastawa	Cucumber	5	SU.26	Bazian	Cauliflower	5
ER.12	Mastawa	Cucumber	2	SU.27	Bazian	Cucumber	1
ER.13	Mastawa	Zucchini	3	SU.28	Bazian	Tomato	1
ER.14	Qushtapa	Cucumber	1	SU.29	Piramagron	Cucumber	5
ER.15	Qushtapa	Lettuce	1	SU.30	Piramagron	Cucumber	3
ER.16	Qushtapa	Cucumber	4	SU.31	Piramagron	Arugula	1
ER.17	Qushtapa	Cucumber	5	SU.32	Piramagron	Cucumber	5
ER.18	Pirdawd	Cucumber	2	SU.33	Dokan	Cucumber	1
ER.19	Pirdawd	Cucumber	2	SU.34	Ranya	Tomato	5
ER.20	Pirdawd	Cucumber	3	SU.35	Ranya	Tomato	3

* Letters in the sample codes indicate the location of the samples collected from greenhouses of northern Iraq. DU: Duhok, ER: Erbil, SU: Sulaimani

** (0 = no galling, 1 = trace infection with some minor galls, 2 = < 25% galled roots, 3 = 25-50% of galled roots, 4 = 51-75%, and 5 = > 75% of galled roots)

The galls in different quantity and size were developed all over the plant roots as well as the plants showed symptoms like stunted, chlorosis and wilting. Roots with GI.5 were decayed and only a few roots with large galls remained. Plant roots with GI.1 and GI.2 did not show any above ground symptoms, although root galls were recognizable. In addition, the severity of infection and quantity of galls varied significantly from one sample to another sample. Based on the results of percentage of gall scale in each province, the samples from Sulaimani province were greater severe damage by RKN than that of Duhok and Erbil provinces.

This is the first survey study on the infestation rate of RKN in greenhouses of Sulaimani and Erbil provinces. In Duhok province, a local survey was conducted in greenhouses of four places of Semel counties (Ami et al., 2018) and similar results were obtained. Additionally, an investigation was carried out on eggplant fields of Niynavah province to determine the distribution and infestation rate of RKNs by Ami (2006). The highest infestation rate (51.66%) recorded in Salamia district. In the survey study conducted by Waref et al., (2018). it was reported that highest infestation rate of 100% with average gall index 3 recorded in Al-Zafar Aniyah/ Baghdad in olive tree, while the lowest infestation rate (6.6%) recorded in nurseries under shade condition of the same area.

Figure 4.2. Root galls on infected plants. A=non infection (sample with GI.0); B, sample with GI.1 on tomato; C= tomato sample with GI.2; D= tomato sample with GI.3; E= tomato sample with GI.4; and F= tomato sample with GI.5

The above ground and underground symptoms related with RKNs were clearly showed in all infected plants of 30 different locations from Sulaimani, Erbil and Duhok provinces. the severe infected plants showed symptoms of stunting, chlorosis, loss of yield, patching and death of plant. Simultaneously, the underground symptoms caused by RKNs were obvious, the galls which produced by root-knot nematodes interrupt the vascular system of plant which led to reduce plant growth and death of plant under severe infestation (Dale, 1973; Brandon, et al., 2011).

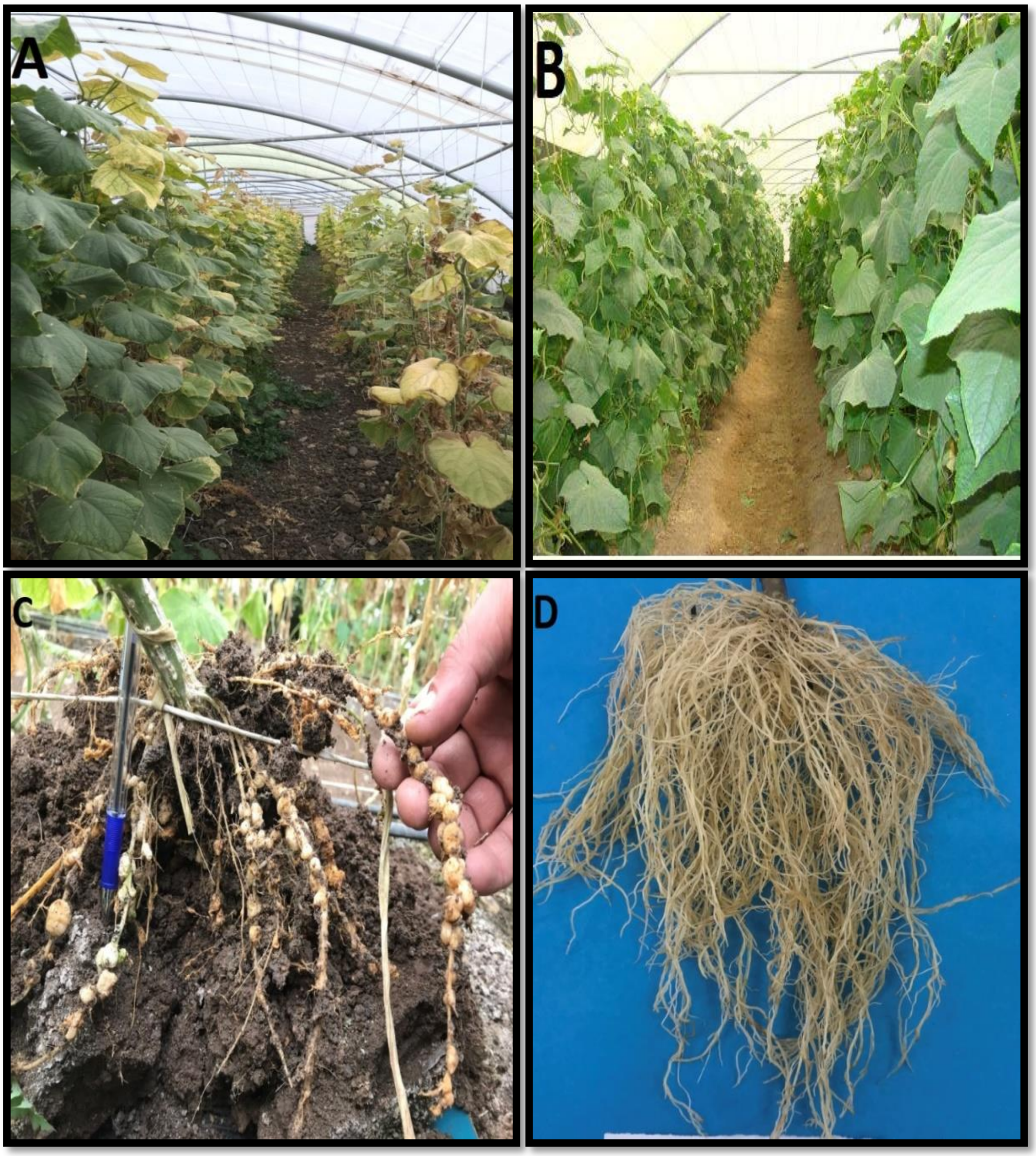


Figure 4.3. Symptoms of RKN on cucumber plants. (A), Above ground symptom (chlorosis) on infected cucumber plants. (B), Healthy cucumber plants in compared with infected plants that showed at A. (C), underground symptom (Gall) of RKNs on cucumber plant. (D), Root system of healthy plant

4.2. Identification of Root-Knot Nematode Populations

Identification of RKN, *Meloidogyne* spp. was carried out based on the morphological and biochemical diagnosis of 70 populations representing different greenhouses. The diagnosis of the species was made by evaluating the results obtained from both methods together.

4.2.1. Morphological Diagnosis of *Meloidogyne* spp.

Morphological identification of root-knot nematodes was prepared based on the procedure for preparing perineal pattern as outlined by Taylor and Netscher, (1974). Ten females were used for identification of perineal patterns in each population. Diagnosis of *Meloidogyne* species based on morphology of perineal pattern was difficult due to the existence of several variations among species (Oliveira, et al. 2011). According to perineal pattern morphology, two RKN species, *M. javanica* (Figure 4.4) and *M. incognita* (Figure 4.5) were identified in surveyed greenhouses of northern Iraq.

The populations were identified based on the main diagnostic feature for identification of *Meloidogyne* species. Forty-five populations were identified as *M. javanica*, 26 from Sulaimani, 13 from Erbil and 6 from Duhok province. Their perineal pattern was easy to separate from another species because of the existence of obvious lateral lines, the pattern structure of this species was oval-shaped or spherical beside low dorsal arch (Aydınlı and Mennan, 2016), which compared with perineal pattern drawing by (Shurtleff and Averre, 2000). In addition, pattern of these species has two lateral line used as a key for separating pattern of *M. javanica* from another species of *Meloidogyne* (Taher et al. 2012). Whereas twenty-five population showed pattern of *M. incognita*, 9 from Sulaimani, 7 from Erbil and 9 from Duhok province, their perineal pattern distinguished by the appearance of high and straight dorsal arch and obscure lateral lines (Aydınlı and Mennan, 2016).

Due to the increasing in the number of species, it has been previously reported that using only genital characters of female perineal pattern for diagnosis will make diagnosis difficult (Whitehead, 1968; Eisenback et al., 1981). Carneiro et al. (2004) stated that using only perineal pattern characters to identify root-knot nematode species in coffee plantations may cause misdiagnosis. It is known that *M. paranaensis* was diagnosed as *M. incognita* for 22 years by using only the morphological characters of genital area and the host test for diagnosis (Carneiro et al., 1996). Hernandez et al.

(2004) reported that perineal pattern characters will contribute to the identification of species in terms of complementing the results obtained with other diagnostic methods. As mentioned in the literature review in this study, it was understood that it would not be possible to definitively identify the species using only the female perineal pattern characters. In this respect, esterase phenotype was used to diagnosis the populations.

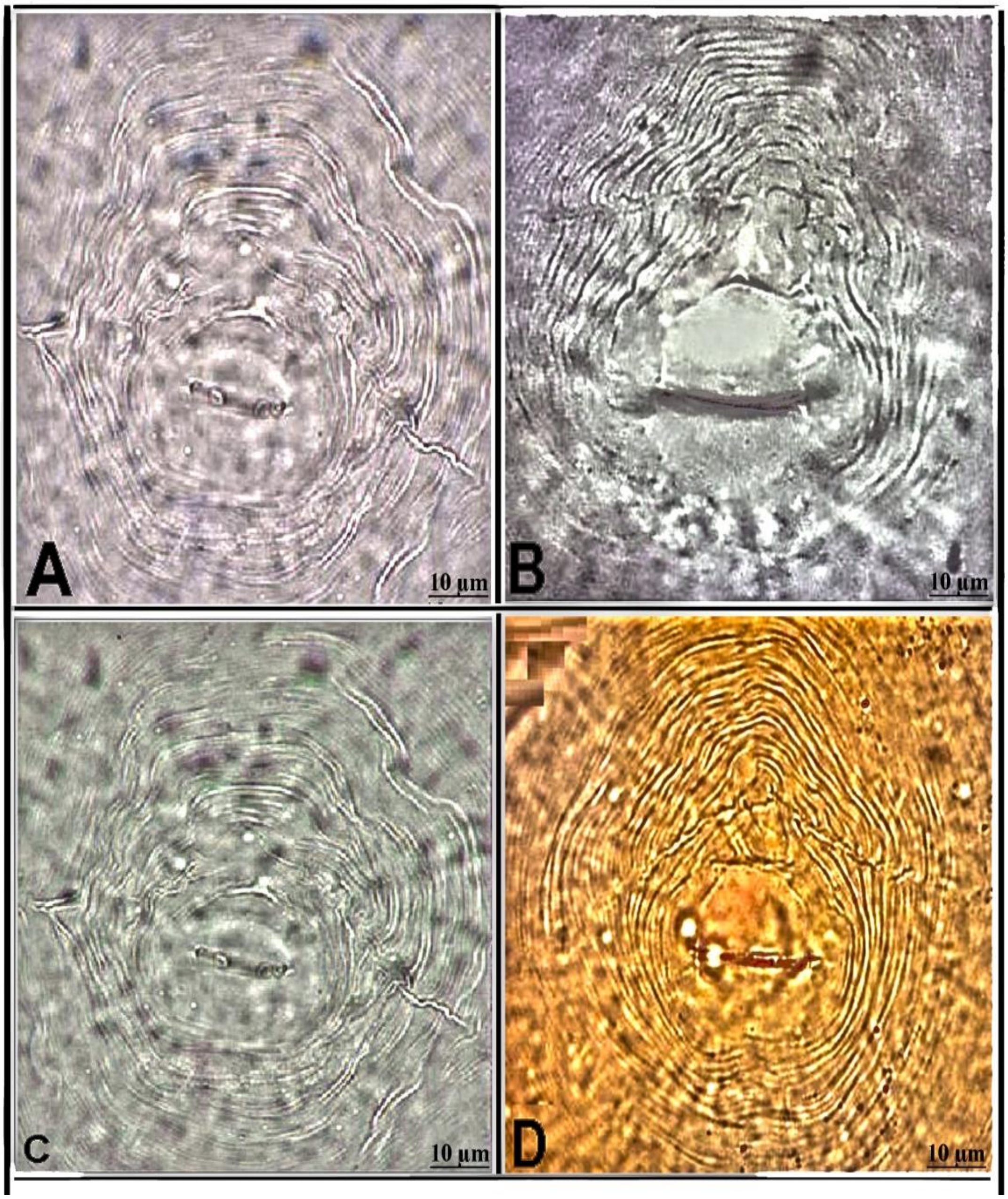


Figure 4.4. Perineal patterns of *M. javanica* from samples (A) DU.4; (B) SU.14; (C) ER.8; (D) SU.29

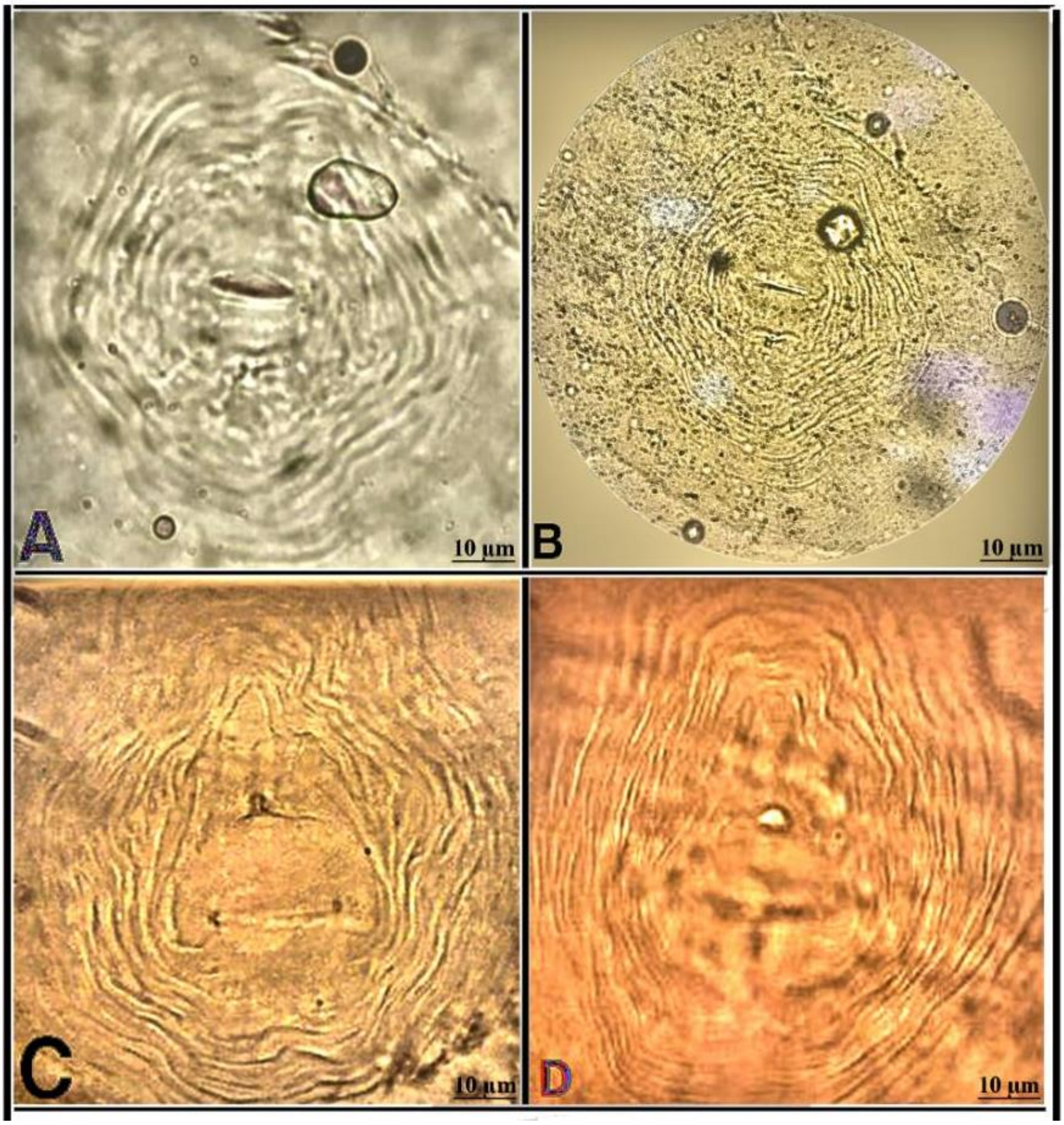


Figure 4.5. Perineal patterns of *M. incognita* from samples. (A) DU.7; (B) SU.27; (C) ER.18; (D) SU.11

4.2.2. Biochemical identification

Esterase phenotype is the most useful character that used in diagnosis of RKN species (Blok and Powers, 2009). In Iraq, diagnosis of *Meloidogyne* species by esterase phenotypes has not been detected previously. Identification based on the esterase phenotype (EST) is usually enough and reliable for diagnosis of *Meloidogyne* species (Esbenshade and Triantaphyllou, 1990; Carneiro et al., 1996).

In this study three individual esterase phenotypes have been recorded between 70 populations of *Meloidogyne* species. EST J3 (Figure 4.7), was detected in 45 (64.28%) populations, 26 from Sulaimani, 13 from Erbil and 6 from Duhok province. EST I2 (Figure 4.8), detected in 22 (31.42%) populations, of which 9 from Sulaimani, 8 from Duhok and 5 from Erbil provinces. As well as, least found EST I1 detected in 3 (4.28%) populations, 2 from Erbil and 1 from Duhok province. J3 phenotype was detected in 45 of the populations and they were identified as *M. javanica*. I1 and I2 were detected in 25 of the populations and they identified as *M. incognita*. (Table 4.9).

Based on the result of current study J3 has been reported as more frequently detected esterase phenotype of root-knot nematodes in northern region of Iraq. I2 phenotype is the second most common esterase phenotype after the J3 phenotype in this region. J3 phenotype belonging to *M. javanica* species used as a reference in esterase enzyme phenotype studies, the researchers who conducted the first studies on esterase enzyme phenotypes stated that all populations of *M. javanica* have a single phenotype (J3) and can be used reliably to distinguish *M. javanica* from other species (Esbenshade and Triantaphyllou, 1985; Fargette, 1987b; Pais and Abrantes, 1989).

The esterase phenotype is usually sufficient to correct identification of root-knot nematode species (Esbenshade and Triantaphyllou, 1985; Fargette, 1987a, 1987b; Pais and Abrantes, 1989; Fargette and Braaksma, 1990; Carneiro et al., 2000; Brito et al., 2008). This is the first study that used esterase phenotype for identification of *Meloidogyne* spp. in Iraq.

Table 4.9. Distribution of EST phenotypes of RKN populations detected in greenhouses in the northern region

Provinces	Number populations	Total number of infected samples	J3		I2		I1	
			Number of infected samples	Average (%)	Number of infected samples	Average (%)	Number of infected samples	Average (%)
Sulaimani	88	35	26	74.28	9	25.71	0	0
Erbil	59	20	13	65	5	20	2	10
Duhok	40	15	6	40	8	53.33	1	6.66
Total	187	70	45	64.28	22	31.42	3	4.28

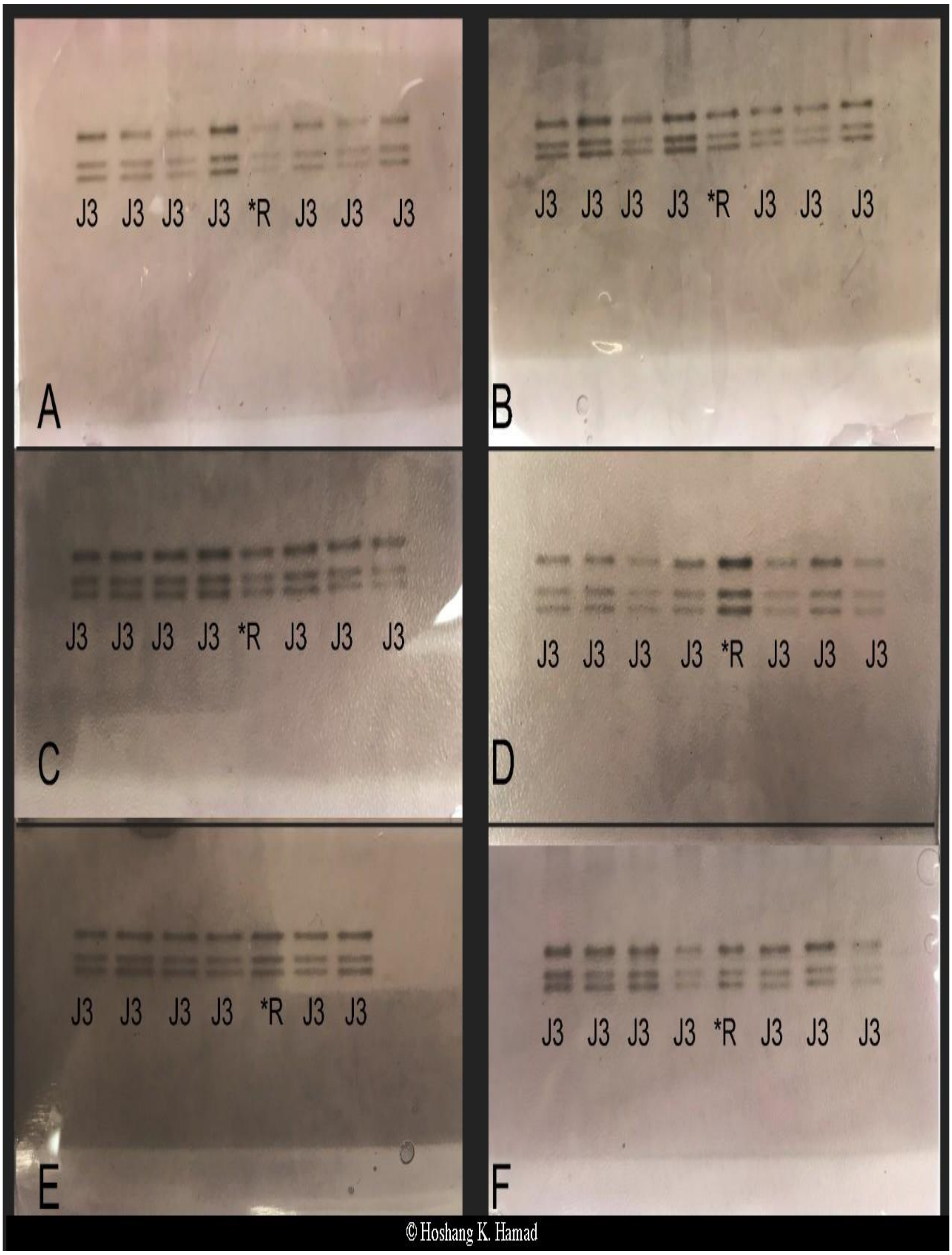


Figure 4.6. Esterase phenotype of *M. javanica*. Each well represents a single female from each population. (A), ER.2; (B)ER.19; (C)DU.9; (D) DU.3; (E) SU.23; (F) SU.35 (*R = Refrence sample, *M. javanica*)

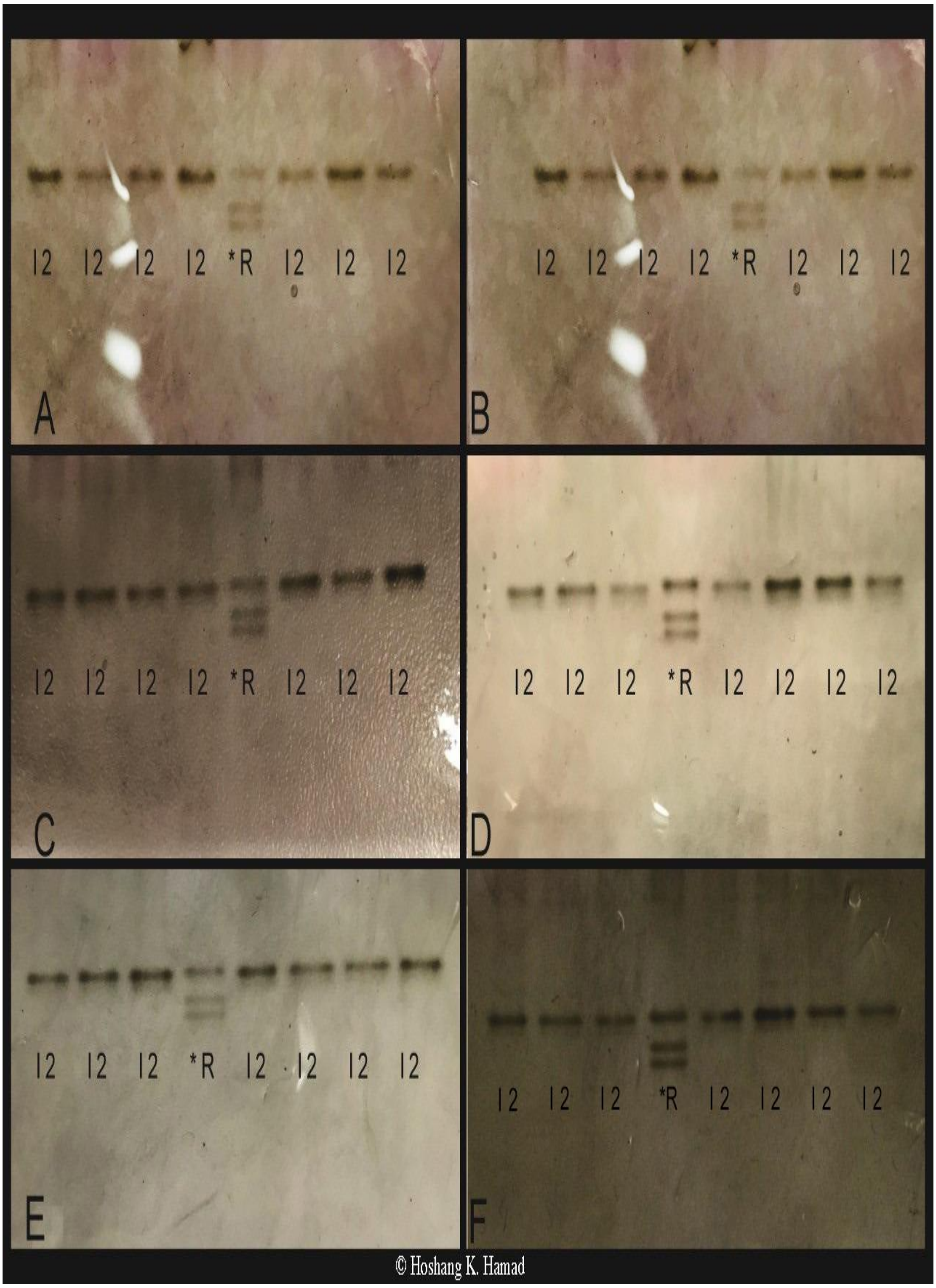


Figure 4.7. Esterase phenotype of *M. incognita*. Each well represents a single female from each population. (A), DU.1; (B), DU.5; (C) ER.6; (D) SU.27; (E) SU.34; (F) ER.13 (*R = Refrence sample, *M. javanica*)

4.2.3. Results of morphological and biochemical identification

The types of the populations were determined by evaluating the results obtained from the genital area morphology and esterase phenotype study together. Of the 70 populations used in the study, 45 were detected as *M. javanica* (64.28%) and 25 *M. incognita* (35.71%). The morphology of the genital areas belonging to the *M. javanica* species is easily distinguished from the others. The populations with genital areas of this species have been identified in Sulaimani, Erbil and Duhok provinces. Esterase phenotype of 45 populations with genital area belonging to *M. javanica* was determined as J3. As well as, population with genital area of *M. incognita* identified in 25 population. Esterase phenotype of 22 populations with genital area morphology belonging to *M. incognita* was determined as I2 and 3 populations as I1.

Based on the both methods of identification, *M. javanica* was detected in 26 (74.28%) greenhouses in Sulaimani, 13 (65%) greenhouses of Erbil and 6 (40%) greenhouses of Duhok province. *M. incognita* was detected in 9 (25.71%) greenhouses in Sulaimani, 7 (35%) greenhouses of Erbil and 9 (60%) greenhouses of Duhok province (Table 4.10). In current study *M. javanica* was determined as most widespread nematode species in the vegetable-grown greenhouses of Sulaimani province as detected in 26 greenhouses in which tomato, cucumber, eggplant, arugula, cauliflower, and zucchini were cultivated, whereas *M. incognita* is the most widespread species detected in 9 greenhouses in Duhok province (Figure 4.9).

M. javanica has been the first report of root-knot nematodes detected in Iraq. It was identified based on the female perineal pattern by (AL_Adhami, 1955). The presence of root-knot nematodes in this region has been reported in surveys conducted at various times by several researchers, as a result of the diagnostic studies based on the characteristics of female perineal pattern morphology, it was found that two species of *Meloidogyne* including, *M. javanica* and *M. incognita* are common species in Iraq (Stephan, 1991; AI-Sabie and Ami, 1992; Ali et al., 2014; Luay and Riadh, 2014; Sulaiman et al., 2018).

Similar study was carried out in Duhok province by Ami et al., (2018). They identified *M. javanica* based on the morphological and molecular techniques in four different places of Semel districts. Furthermore, based on the results of study in Babylon governorate, central region of Iraq by Hasan and Abood (2018), both species of *M. javanica* and *M. incognita* with rate (73.33% and 20%) identified depending on perineal pattern characters and molecular identification. In addition, all of the populations in current study found singly, no mixed population recorded in this survey study. This research study is the broadest study to detect and identify of *Meloidogyne* species occurring in vegetable grown greenhouses of northern region of Iraq.

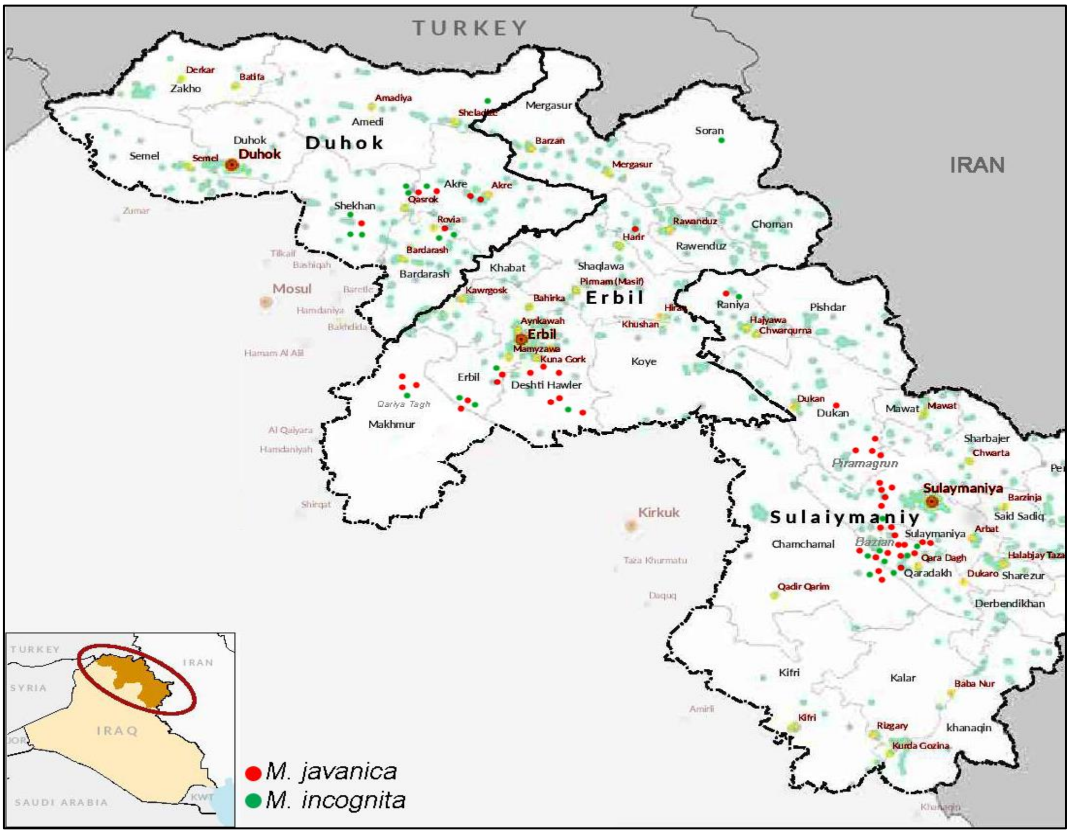


Figure 4.8. Map shows geographic localization of detected *Meloidogyne* spp. in northern Iraq. A single dot represents a single population

Both species of *Meloidogyne* were found on the host plants including cucumber, tomato, zucchini and lettuce from which the population were obtained *M. javanica* is also found in the eggplant, arugula and cauliflower, which are less cultivated than cucumber and tomato. However, *M. incognita* is not detected on eggplant, arugula, and cauliflower (Figure 4.10).

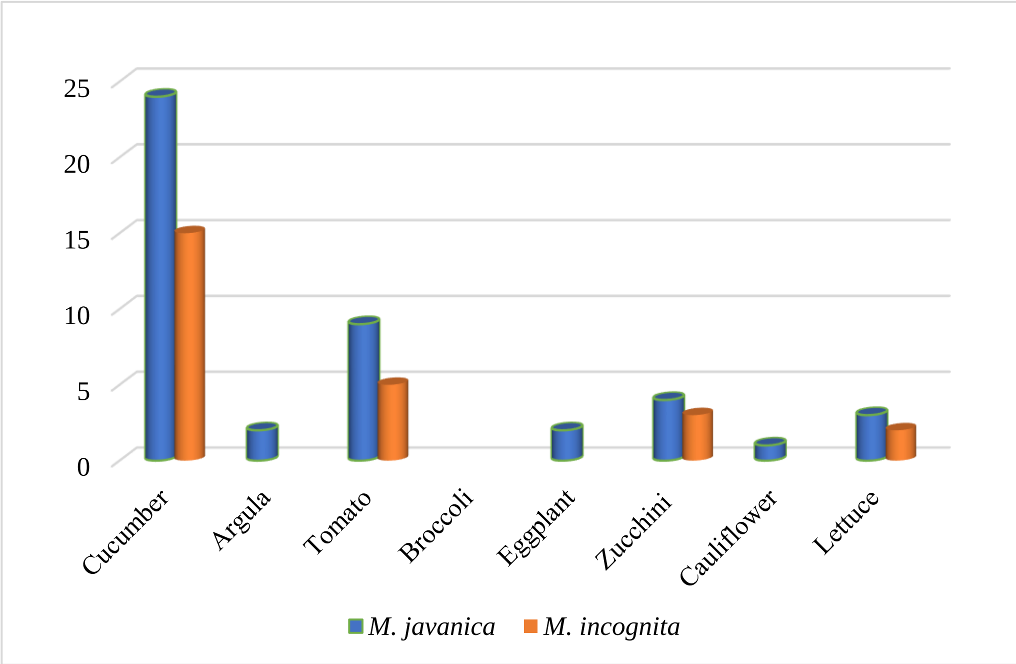


Figure 4.9. Number of populations of *M. javanica* and *M. incognita* in host plants

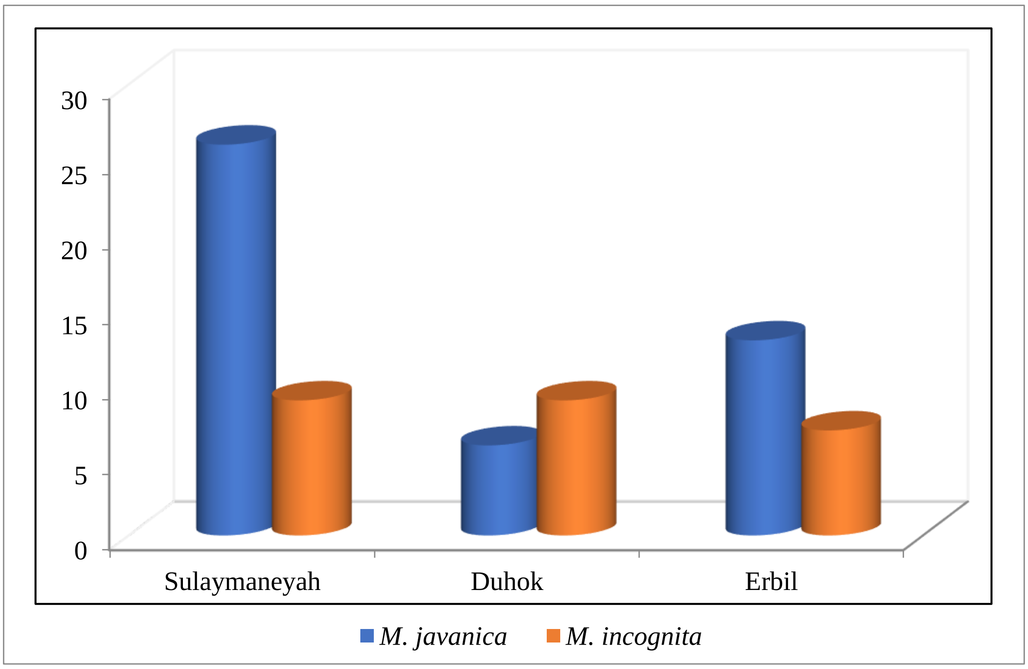


Figure 4.10. Occurrence of *Meloidogyne* spp. in vegetable-grown greenhouses of Sulaimani, Erbil and Duhok provinces, Iraq

Table 4.10. Results of biochemical and morphological identification of 70 populations of RKN collected from greenhouses of Northern Iraq

Population code	Host plants	Identification methods		Species
		Perineal pattern	Esterase phenotype	
DU.1	Tomato	*M. incognita*	I2	*M. incognita*
DU.2	Cucumber	*M. incognita*	I2	*M. incognita*
DU.3	Cucumber	*M. javanica*	J3	*M. javanica*
DU.4	Lettuce	*M. javanica*	J3	*M. javanica*
DU.5	Cucumber	*M. incognita*	I2	*M. incognita*
DU.6	Tomato	*M. javanica*	J3	*M. javanica*
DU.7	Tomato	*M. incognita*	I1	*M. incognita*
DU.8	Cucumber	*M. incognita*	I2	*M. incognita*
DU.9	Cucumber	*M. javanica*	J3	*M. javanica*
DU.10	Cucumber	*M. javanica*	J3	*M. javanica*
DU.11	Zucchini	*M. incognita*	I2	*M. incognita*
DU.12	Cucumber	*M. incognita*	I2	*M. incognita*
DU.13	Cucumber	*M. javanica*	J3	*M. javanica*
DU.14	Tomato	*M. incognita*	I2	*M. incognita*
DU.15	Tomato	*M. incognita*	I2	*M. incognita*
ER.1	Cucumber	*M. incognita*	I2	*M. incognita*
ER.2	Cucumber	*M. javanica*	J3	*M. javanica*
ER.3	Cucumber	*M. incognita*	I2	*M. incognita*
ER.4	Cucumber	*M. javanica*	J3	*M. javanica*
ER.5	Tomato	*M. javanica*	J3	*M. javanica*
ER.6	Tomato	*M. incognita*	I1	*M. incognita*
ER.7	Tomato	*M. incognita*	I2	*M. incognita*
ER.8	Lettuce	*M. javanica*	J3	*M. javanica*
ER.9	Lettuce	*M. javanica*	J3	*M. javanica*
ER.10	Cucumber	*M. javanica*	J3	*M. javanica*
ER.11	Cucumber	*M. javanica*	J3	*M. javanica*
ER.12	Zucchini	*M. javanica*	J3	*M. javanica*
ER.13	Cucumber	*M. incognita*	I1	*M. incoginta*
ER.14	Lettuce	*M. javanica*	J3	*M. javanica*
ER.15	Cucumber	*M. javanica*	J3	*M. javanica*

Table 4.10. (Continue)

ER.16	Cucumber	*M. javanica*	J3	*M. javanica*
ER.17	Cucumber	*M. incognita*	I2	*M. incognita*
ER.18	Cucumber	*M. incognita*	I2	*M. incognita*
ER.19	Cucumber	*M. javanica*	J3	*M. javanica*
ER.20	Cucumber	*M. javanica*	J3	*M. javanica*
SU.1	Tomato	*M. javanica*	J3	*M. javanica*
SU.2	Tomato	*M. incognita*	I2	*M. incognita*
SU.3	Cucumber	*M. incognita*	I2	*M. incognita*
SU.4	Cucumber	*M. javanica*	J3	*M. javanica*
SU.5	Cucumber	*M. incognita*	I2	*M. incoginta*
SU.6	Zucchini	*M. javanica*	J3	*M. javanica*
SU.7	Cucumber	*M. javanica*	J3	*M. javanica*
SU.8	Cucumber	*M. javanica*	J3	*M. javanica*
SU.9	Cucumber	*M. javanica*	J3	*M. javanica*
SU.10	Cucumber	*M. incognita*	I2	*M. incognita*
SU.11	Cucumber	*M. incognita*	I2	*M. incognita*
SU.12	Eggplant	*M. javanica*	J3	*M. javanica*
SU.13	Cucumber	*M. incognita*	I2	*M. incognita*
SU.14	Cucumber	*M. incognita*	J3	*M. incognita*
SU.15	Cucumber	*M. javanica*	J3	*M. javanica*
SU.16	Tomato	*M. javanica*	J3	*M. javanica*
SU.17	Cucumber	*M. javanica*	J3	*M. javanica*
SU.18	Tomato	*M. incognita*	I2	*M. incognita*
SU.19	Cucumber	*M. javanica*	J3	*M. javanica*
SU.20	Cucumber	*M. javanica*	J3	*M. javanica*
SU.21	Arugula	*M. javanica*	J3	*M. javanica*
SU.22	Eggplant	*M. javanica*	J3	*M. javanica*
SU.23	Cucumber	*M. javanica*	J3	*M. javanica*
SU.24	Cucumber	*M. javanica*	J3	*M. javanica*
SU.25	Zucchini	*M. javanica*	J3	*M. javanica*
SU.26	Cauliflower	*M. javanica*	J3	*M. javanica*
SU.27	Cucumber	*M. incognita*	I2	*M. incognita*
SU.28	Tomato	*M. javanica*	J3	*M. javanica*

Table 4.10. (Continue)

SU.29	Cucumber	*M. javanica*	J3	*M. javanica*
SU.30	Cucumber	*M. javanica*	J3	*M. javanica*
SU.31	Arugula	*M. javanica*	J3	*M. javanica*
SU.32	Cucumber	*M. javanica*	J3	*M. javanica*
SU.33	Cucumber	*M. javanica*	J3	*M. javanica*
SU.34	Tomato	*M. incognita*	I2	*M. incognita*
SU.35	Tomato	*M. javanica*	J3	*M. javanica*

5. CONCLUSION AND RECOMMENDATIONS

The study is the most detailed and widest work on determining distribution and infestation rate of RKN species in greenhouses grown vegetable crops of northern region of Iraq. Although 187 composite samples collected from 30 districts of Sulaimani, Erbil and Duhok provinces, seventy (37.40%) of greenhouses was find out to be infested with root-knot nematode species. The highest infestation rate of RKNs were found in Sulaimani (39.77%) followed by Duhok and Erbil provinces with (37.50% and 33.89%), respectively. Among this province, the average of root gall in Sulaimani (3.085) was greater than that of Duhok (2.93) and Erbil (2.6). Among vegetables, the most of the greenhouses of region cultivated with cucumber and the highest infestation rate of RKN was on cucumber (58.33 %) followed by tomato (33.33%), eggplant (25%), arugula (22.22%), zucchini (21.05%), lettuce (20%), and cauliflower (14.28%).

In this study, two species of *Meloidogyne* were identified in all surveyed locations, which include *M. javanica*, and *M. incognita* with 64.28% and 35.71%, respectively. *M. javanica* was determined as a prevalent root-knot nematode species detected in 45 greenhouses in which tomato, cucumber, eggplant, zucchini, lettuce, and arugula plants were cultivated. *M. incognita*, which detected in 25 greenhouses collected during survey in Northern Iraq. Based on the both methods of identification, *M. javanica* recorded as the most predominant species of RKNs identified in 26 populations in Sulaimani province, which is the centre of greenhouse cultivation in Iraq. However, *M. incognita* is the most prevalent species in Duhok province.

In this study, esterase phenotype for identification of RKNs was used for the first time in Iraq. The previous studies were mostly conducted in small scale farming and identified species based on the female perineal patterns and molecular (PCR) identification. Carneiro et al. (1996) in a study revealed that accurate identification of RKN species based only on perineal patterns of an adult female was considerably difficult. RKNs morphologically are quite similar to each other and frequently more than a species of root-knot nematodes found on the same root of infected plants.

The number of sample and identification approaches used in this study in comparison with previous studies includes a more consistently and widely sampling number by taken 187 composite samples from 30 different districts of 3 provinces in northern Iraq. Moreover, esterase phenotypes were used for the first time for identification of RKN populations in Iraq in additional to morphological identification. Twenty-one individual females from each population were used for analysis of their esterase phonotypes and three distinct esterase phenotypes including J3, I2 and I1 were detected among populations. Esterase phenotypes J3 is species-specific for *M. javanica*, while I1 and I2 are M. incognita. When the results obtained with perineal patterns and esterase phenotypes were evaluated together, out of the 70 RKN populations, 45 *M. javanica* (64.28%) and 25 M. incognita (35.72%) were identified.

In conclusion, identification of root-knot nematode species based only on the perineal pattern (future characteristic of adult female) is not sufficient for species identity and time-consuming and needs more skills. However, perineal patterns of RKNs are still frequently used to support biochemical and molecular identification of species. Perineal pattern identification is also necessary for the identification of new species. On the other hand, esterase phenotype is an accurate and reliable method that can be used for the identification of species. Accurate and rapid identification of species is important for establishing appropriate management strategies. The presence of RKNs in greenhouse-grown vegetable zones of northern Iraq is a great value, it causes considerable losses on both quality and quantity of vegetables. Hence, developing management approaches are of great importance for reducing their damage.

Management of RKNs is hard due to the existence of their broad host plants. As an illustration, the majority of vegetable producers utilize nematicide for control of nematodes in the Bazian district, which has the most intensive vegetable production area in Iraq but the area has the highest infestation level caused by RKNs yet. Integrated pest management strategies such as crop rotation, solarization, plant resistance, biological control should be used for RKNs, because it is difficult to control RKN based on only a management tool (Barker et al., 1985).

In Iraq, root-knot nematodes are cause significant vegetable losses. Therefore, integrated pest management should be developed in order to reduce their damage. As well as, the farmers should be awareness about their presence to decrease spread of nematodes and their losses can be minimizing. Correct identification of the species is the first step to being successful in controlling root-knot nematodes. In addition, it should not be forgotten that the populations of the same species in different regions may differ in their damage potential towards host plants. Local control programs to be applied according to the characteristics of the population will help in management of RKNs and ensure the highest yield per unit area.

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