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EXISTENCE, MOLECULAR DIAGNOSIS AND GENETIC VARIATION OF DIFFERENT ISOLATES OF ROOT – KNOT NEMATODES *Meloidogyne* spp. PARASITIZING CUCUMBER PLANTS

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ABSTRACT

The findings indicated that the highest disease incidence (D.I.) was observed in autumn 2021 on cucumber plants grown in the greenhouses of Qustapa, with a rate of 87.5%. This was followed by a D.I. of 70% in the same location during spring 2022 and in the greenhouses of Daraband during autumn 2021.In both growing seasons in Daraband, and in Qustapa during Autumn 2021 more than 100 galls per root system were recorded, indicating a high level of infection and a highest root – knot index (5) . In contrast, no infection (D.I. 0.0%) was found in the greenhouses of Kamosak (Grd Goran) in both growing seasons and in Mastawa location during spring 2022. The results of soil analysis conducted in the surveyed locations revealed that the physical and chemical properties of the soil influence the movement and survival of nematodes. Results of Molecular diagnosis using two universal primers included 10 Picomoles (pmol) of forward primer-TW81 (5'GTTTCCGTAGGTGAACCTGC-3') and 10 pmol reverse primer-AB28 (5'ATATGCTTAAGTTCAGCGGGT-3') identified four species of root-knot nematodes *Meloidogyne* spp., prevalent in cucumber greenhouses across the surveyed locations.

namely. *M. incognita, M. javanica, M. arenaria and M. hapla* and according to their genetic variation two races for each of the first three species were recorded for the first time by this technique.

KEYWORDS: *Meloidogyn*e spp. Molecular diagnosis, Cucumber

INTRODUCTION

Cucumber (*Cucumis sativus* L.(Cucurbitaceae) is one of the most popular vegetables. It is distinguished by high medicinal and nutritional value (Arnaot, 1980). It contains more than 95% water and trace of proteins, vitamins , carbohydrates, fatty acids, minerals, pectins, essential oil, terpenoids and steroids (Kumar *et al.,* 2010). Root-knot nematodes *Meloidogyn*e spp.causing of root – knot disease occur throughout the world, and they attack more than 2,000 species of plants (Agriose,2005).It was discovered that certain root – knot nematode species are the most economically damaging pathogen on cucumber plants in both greenhouses and fields and causing up to 50% plant damage (Katooli *et al.*,2010).The four root –knot nematode species that inflict the most significant economic harm to various crops are *M .javanica, M. incognita, M .arenaria* and *M. hapla* (Khalil, 2013). Species of *Meloidogyne* have been diagnosed depending on their morphological characteristics (Eisenback and Triataphyllou,1991), host -plant response (Hartman and Sasser,1985), Isozyme analysis (Esbenshade and Triantaphyllou,1990) and molecular techniques (Ziljistra, *et al.*, 1995).

Application of molecular techniques in plant pathology has greatly improved specialists ability to detect plant pathogens (Martin *et al.*,2000).There are several causes that confirm why molecular information is more trustworthy for phylogenetic analysis than morphological traits. DNA sequences are firmly irritable and the interpretation of molecular information is more accurate than morphological characteristics. In addition, molecular traits are more plentiful and are generated over shorter period of time (Subbotin and Moens, 2006) .When compared to morphological or biochemical techniques, Molecular techniques are known to be more dependable, sensitive, robust, and specific in detecting and distinguishing numerous *Meloidogyne* species

(Powers *et al.*,2005 and Berry *et al.*,2007).Molecular diagnosis of nematode can be conducted using a group sample of J2s and females or single J2s (Blok, 2005) or nematode eggs.

This study aims to verify the existence of root-knot nematodes *Meloidogyne* species on cucumber plants in some areas of Erbil province, Kurdistan Region – Iraq, and the use of molecular technique in the diagnosis of all isolates (populations) of this nematode genus that will be extracted from infected cucumber roots after collecting them during a field survey of plastic greenhouses.

MATERIALS AND METHODS

1. Survey of greenhouses planted with cucumbers in Erbil province for detection of root – knot disease

Survey was conducted in five locations within Erbil province including: Qushtapa, Grdgoran (Kamusak), Daraban, Khabat and Mastawa by choosing three greenhouses in which cucumber was planted in each location during the months of August, September and October in 2021 and the months of May, June, and July in 2022. Cucumber roots were examined to ensure that the plants were infected with root-knot disease or not, where the infected plants were characterized by wilting, stunting, chlorosis and death in severe infection compared to the healthy plants (Figure,1). According to Coyne *et al.,* (2007).Systemic pattern (zigzag technique) (on alternations of planting lines) was used for root and soil sampling. Each greenhouse was considered as a single unit and the following characters were calculated:

-A- -B- -C-

Fig. (1):-Infected cucumber plants with different disease severity (A and B) compared to the healthy plants (C)

1.1.Disease incidence (D.I.) (%): The calculation was performed by examining cucumber roots and ensuring the presence of galls on them (Figure,2) and the following equation was used for calculation of disease incidence (Kayani *et al.*, 2012).

(D.I) (%) = (No. of infected plants / Total No. of examined plants) × 100

1.2- Disease severity (D.S.) : Disease severity or root gall severity was calculated depending on root – knot index which consists of 0-5 scales and as follows: 0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31- 100 and 5 more than 100 galls (Kumar *et al* ., 2014)

1.3. Experimental design : The survey experiment is set up as factorial experiment in Completely Randomized Design (CRD) and comprises of 10 treatments (5 locations × 2 growing seasons) with 120 replications (3 greenhouses × 40 plants in each location). Data were analyzed and Duncan's multiple range test, $p \leq 0.05$ was used for comparison between the means of disease incidence in the surveyed locations as mentioned by Al –Rawi and Khalafa Allah,(1980) and Al- Zubaidy and Al – Falahy (2016).

Fig. (2) : -Galls on the roots of cucumber caused by root-knot nematodes *Meloidogyne* spp.

1.4. Collection of soil and root samples: Roots of the diseased cucumber plants and Soil samples were taken from greenhouses keeping in mind that the samples were representative to an area for each greenhouse. Garden trowel and garden spade were utilized for soil sampling to a depth of 20 – 30 cm at which cucumber roots could be easily checked. Infected root samples and soil for each greenhouse were putted in polyethylene bags . Infected root samples were moistened with little amounts of distilled water, and for each sample planting date, the sampling date and location name were recorded. Samples were saved in a cool boxes (insulated containers) with ice and transported to the laboratory where they were preserved at 4ºC for no more than three days where mature females were extracted to be used for diagnosis species of root – knot nematodes *Meloidogyne* spp.by molecular technique. As for the soil samples, they were analyzed in Agricultural Research Directorate Laboratory, Ain Kawa / Erbil province for determination of their chemical and physical properties, which included:

1.4.1. Soil texture : It was determined by a hydrometer method according to Jaiswal (2003) to estimate the percentage of soil content of sand , silt and clay.

1.4.2. Soil pH : It was determined by pH meter model Hans Herbert Mennerich (geotechnik) Hanover as clarified by Van Reeuwijk (1995).

1.4.3. Electrical Conductivity (E.C.) : It was estimated by using EC meter ,model D8120 and

adjusted to 25ºC as mentioned by Van Reeuwijk (1995).

1.4.4. Organic carbon percentage : it was measured in the soil by wet digestion method with concentrated sulphuric acid (Walkley and Black method) according to Black (1965).

1.4.5. **Percentage of Organic Matter (OM %)** = Total organic carbon (%) x 1.72

1.4.6. Total Nitrogen : Its estimation was done according to Kjeldhal method as described by Rowell (1996).

1.4.7. C / N ratio: It was calculated by dividing organic carbon (%) over total nitrogen (%).

2. Molecular diagnosis of different isolates of root – knot nematodes *Meloidogyne* spp. parasitizing cucumber plants:

2-1. Extraction of mature females of *Meloidogyne* spp. : For all root-knot nematode isolates mature females (Figure,3) were removed from infected cucumber roots collected during a field survey of plastic greenhouses in different locations of Erbil province included three isolates from each of Mastawa, Qushtapa, Daraban and one isolate from Khabat location. This process was done in Lab.22, Molecular genetics of animal resource / College of Agricultural Engineering Sciences / University of Salahaddin - Erbil/ Kurdistan Region – Iraq.

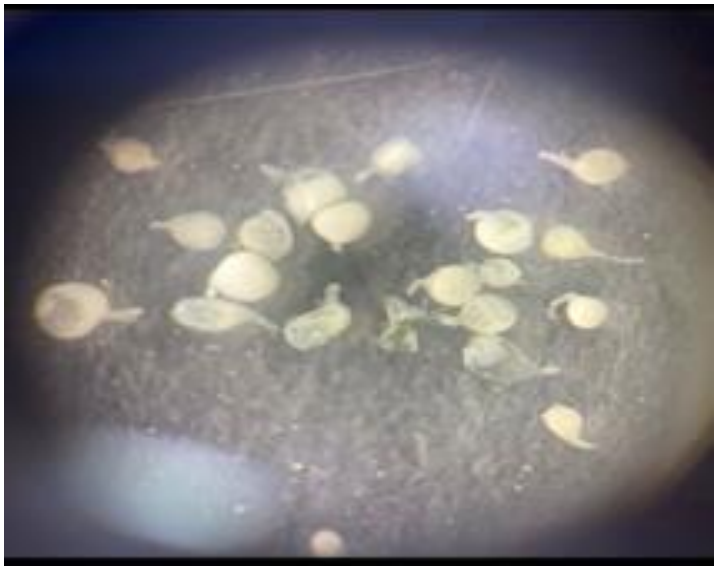

Fig. (3) :- Mature females of root – knot nematodes *Meloidogyne* spp.

2-2. DNA extraction and purification: For each of the 10 isolates of root – knot nematodes *Meloidogyne* spp**.** Genomic DNA was separately extracted from several mature females according to Beta Bayern tissue DNA preparation Kit (Beta Bayern GmbH .90453 Bayern, Germany) method using Worm Lysis Buffer (WLB), as explained by Castagnone - Sereno *et al.*, (1995). Sample transferred to a new tubes of PCR which were cooled on an ice to be centrifuged at 6000 rpm for half a minute. **(** Waeyenberge *et al*, 2000).The supernatant material that contains DNA was collected and saved at -20 °C as used by Onkendi and Moleleki, (2013) to be amplified later using Polymerase Chain Reaction (PCR)..

2-3. Amplification of the target DNA by Polymerase chain reaction (PCR): For amplification of the internal transcribed spacer (ITS rDNA) region, which contains ITS1, 5.8S rRNA coding sequence, and ITS2 (Skantar *et al*,2012), two universal primers were used. These included: 10 Picomoles (pmol) of forward primer-TW81(5’GTTTCCGTAGGTGAACCTGC-3’) and 10 pmol reverse primer-AB28 (5’ATATGCTTAAGTTCAGCGGGT-3’), both of which were provided by Germany company (Jena Bioscience). The amplifications were done as described by Michel and Sikora (2005). On 1.5 % agarose gel stained with Gel Red in 1 × TAE, The amplified products were separated from a phiX174 DNA / HaeIII marker before being examined under UV light by UV Trans Illumination.

3. SEQUENCING OF DNA

3-1. Clearing of sequences results ; Sequencing of the ten samples of PCR product 5.8S rRNA partial gene were done using ABI Prism Terminator Sequencing Kit (Applied Biosystem) at Macrogen Molecular Company of Korea to determine the order of the 4 nitrogenous base, including: Adenine (A), Guanine(G), Cytosine (C) and Thymine (T) in the strand of DNA. Using FinchTV program software, chromatograms of 5.8S rRNA gene were edited and base calls were verified.

3-2. Sequence alignment : Following the submission of the sequences of the ten isolates of *Meloidogyne* spp. to Gen-Bank at NCBI, the accession number was determined as follows: ON833423.1, ON833424.1, ON833425.1, ON8334261, ON833427.1, ON833428.1, ON833429.1, ON833430.1, OQ693824.1 and OQ693825.1 for each of nematode isolates included 1Ma1.E.,1 Ma2,E., 1Ma3.E, 2Qu1.E., 2Qu2.E. 2Qu3.E, 3 Kh. E., 4 Da1. E., 4 Da2. E and 4 Da3. E respectively collected from different locations in Etrbil province included: Mastawa, Qustapa, Khabat and Daraban. The 5.8S rRNA gene sequences were applied to Basic Local Alignment Search Tool (BLAST) which is a search tool that uses the sequence alignment method and is available at the NCBI (National Center for Biotechnology Information) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for extraction percent identity for the ten nematode isolates and by which genetic variability and affinity among nematode species and isolates diagnosed in this study can be compared with each other and with the other sequences for species and isolates selected by NCBI.

4. Extraction of DNA Dot Plot: It is a graphical method available in NCBI BLAST program as mentioned previously for comparing two biological sequences with each other and used as another method for comparing sequences of the ten nematode isolates with each other and with the other sequences for species selected by NCBI.

RESULTS AND DISCUSSION

1. Survey of greenhouses planted by cucumber plants in Erbil province for detection root-knot disease :

Results of statistical analysis indicated that the interaction between surveyed locations and growing seasons was significant in its effect on the infection criteria (Table,1). From the same table the findings indicated that during the autumn season, the highest D.I reached 44.5%, and the root systems exhibited the highest number of galls, measuring 83.8 galls. These results were significantly different from those observed in the spring season. Furthermore, the autumn season recorded the highest root-knot index, measuring 3.47, which was significantly different from the lowest value of 2.5 observed in the spring season.

Table (1) :- Infection criteria of infected cucumber plants in greenhouses of surveyed locations in both growing seasons

Locations	Infection criteria *					
	Disease Incidence (%)		The highest number of galls / root system		Root – knot index / root system	
	Autumn 2021	Spring 2022	Autumn 2021	Spring 2022	Autumn 2021	Spring 2022
Daraban	70 b	60 c	161 ab	163 a	5 a	5 a
Qushtapa	87.5 a	70 b	154 b	81 c	5 a	4.5 a
Kamosak (Grd Goran)	0.0 e	0.0 e	0.0 e	0.0 e	0.0 c	0.0 c
Khabat	10 d	12.5 d	17 d	18 d	3 b	3 b
Mastawa	55 c	0.0 e	87 c	0.0 e	4.33 a	0.0 c
Effect of the growing season	44.5 a	28.5 b	83.8 a	52.4 b	3.47 a	2.5 b

- For the effect of the growing seasons each number is a mean of 15 values (5 locations x 3 replications – greenhouses-).

* Numbers for each infection criteria for the effect of the interaction or growing season followed by different letter significantly differ according to the Duncan's Multiple Range Test ($p \leq 0.05$) .

Regarding the impact of the surveyed locations, Qushtapa exhibited the highest D.I at 78.75%, significantly differing from the other locations. Conversely, Daraban showed the highest number of galls, with 162 galls per root system, significantly different from the other locations. Among all the locations, Daraban also reported the highest root-knot index of 5, which

significantly differed from the other locations except for Qushtapa location with significant difference as compared to the other locations except Qustapa, meanwhile no infection was found in Kamosak (Grd Goran) location.(Table,2) .

Table (2) : -Infection criteria as affected by surveyed locations

Locations	Infection criteria *		
	Disease incidence (%)	The highest number of galls / root system	Root – knot index / root system
Daraban	65 b	162 a	5 a
Qushtapa	78.75 a	117.5 b	4.75 a
Kamosak (Grd Goran)	0.0 e	0.0 e	0.0 d
Khabat	11.25 d	17.5 d	3 b
Mastawa	27.5 c	43.5 c	2.165 c

- Each number is a mean of 6 values (2 growing seasons x 3 replications – greenhouses -).
* Numbers followed by different letter for each infection criteria significantly differ according to the Duncan's Multiple Range Test ($p \leq 0.05$) .

2. Physico-chemical properties of the soil in the surveyed locations : Results showed that 53.85% of the soil of the surveyed greenhouses were clay loam and 30.77% were clay soil .The maximum percentage of Clay (51.5 %) was found in the soil of one of the greenhouses of Kamoosak location, while the lowest (22.4%) was reported in one of the greenhouse of Khabat location where its soil texture is sandy clay loam. The highest percentage of the sand (52.4%) was recorded in the soil of one of the greenhouses of Khabat location, however the lowest (15.8 %) was observed in the soil of one of the greenhouses of Kamoosak location..The highest percentage of organic matter (2.23 %) was reported in one of the greenhouses of Qustapa and the lowest (1.07%) in another greenhouse of the same location. Soil EC increased in the soil of one of the greenhouses of each of Mastawa and Kamoosak to be 5.6 and 5.5 $_{Dsm}$-1 respectively, which return back to various reasons, including high surface evaporation, irrigation with saline water and poor cultural practices (Jamil, *et al.*, 2011) or may be due to insufficient leaching of ions from the soil profile at the time of soil sampling, then declined to 1.1 $_{Dsm}$-1 in as a minimum value in one of the greenhouses of Qustapa. The results of pH were almost similar in all locations ranging from 7.88- 8.3. In general C/N ratio in all surveyed soils is less than the optimum ratio C/N which is 24:1 (https://advancecovercrops.com/resources-advanced-cover-crops/carbon-nitrogen-ratio),however, it appears from the results that the highest C/N ratio (7.20) was recorded in one of the greenhouses of Qustapa, while the lowest (1.42) in another greenhouse in Mastawa (Table,3).

Table (3) :- Physic - chemical properties of soils in the surveyed locations.

Location	Green - house	Clay %	Silt %	Sand %	Soil texture	Organic matter %	E C $_{Dsm}$-1	pH	C / N ratio
Daraban	1	27.4	45.2	27.4	Clay loam	1.45	3.1	8.5	7.03
Qushtapa	1	44	27.7	28.3	Clay	1.07	1.5	8.18	3.66
	2	36.5	32.7	30.8	Clay loam	2.23	3.4	7.88	7.20
	3	39	32.7	28.3	Clay loam	1.96	1.1	8.08	2.03
Kamosak (Grd Goran)	1	51.5	32.7	15.8	Clay	1.23	2.2	8.07	2.86
	2	44	35.2	20.8	Clay	1.47	5.5	7.95	4.50
	3	44	35.2	20.8	Clay	1.36	3.2	7.93	2.47
Khabat	1	22.4	25.2	52.4	Sandy clay loam	1.08	2.2	8.12	3.14
	2	37.4	42.7	19.9	Silty clay loam	1.66	2.7	8.09	4.60
	3	29.9	42.7	27.4	Clay loam	1.46	3.5	8.15	3.26

Mastawa	1	39.9	37.7	22.4	Clay loam	1.32	2.1	7.94	1.42
	2	32.4	37.7	29.9	Clay loam	1.66	1.6	8.13	2,61
	3	32.4	42.7	24.9	Clay loam	1.94	5.6	7.81	4.70

E.C = Soil electrical conductivity (Measure of soil salinity)
$_{Dsm}$-1 = DeciSiemens per meter. C = Carbon. N = Nitrogen. pH = Potential of Hydrogen

The prevalence of nematode infection in the surveyed areas can be attributed to specific physical and chemical properties of the soil, particularly its texture. Soil texture plays a significant role in the movement of nematodes (Naz et al., 2012). The study's findings suggest that the soil texture provides favorable conditions for nematode movement, primarily due to the presence of pore spaces that can retain an adequate amount of moisture necessary for the movement of nematode juveniles towards the roots of the host plants.

Additionally, a few percent of clay particles in the soil attracts nematode juveniles from far away to plant roots through adsorption of root exudates on their surfaces, this opinion is consistent with what has been reported by Prot and Van Gundyl (1981).This indicates that nematode migration toward the plant roots and afterwards their reproduction are influenced by the soil texture. (Hussain *et al.*,2012 and Naz *et al.*2012)..Moreover, the other soil properties in all areas including Organic matter, E C and pH were in a suitable range for the presence of root – knot nematodes (Al-ghamdi,2021).This is also confirmed by Ismail (1998) for organic matter and pH, and by Karajeh and Al-Nasir (2014) for E C of the most surveyed soil except in one of the greenhouses of Kamosak and Mastawa locations and it was one of the reasons for decreasing infection criteria in both locations. Olabiyi *et al.,(*2009) indicated also that such pH values are within range necessary for nematode reproduction and survival. The C/N ratio of soils in all surveyed areas is less than 20 (with enough nitrogen) which has a role in reducing nematode populations as reported by Agbenin (2004) and Agu (2008) and depending on the ratio mentioned by Stirling (1991) that C / N ratio which is less than 20:1 has a nematicidal effect. In this case, the higher nitrogen content and low C/N ratio cause the production of toxic ammonia which reduce nematode reproduction in the soil (Rivera and Aballay, 2008; Oka, 2010).Therefore,,the absence of the infection or its decrease in some locations such as Kamoosak and Khabat are respectively may also attributed to the effect of less C/N ratio on nematode activity, while an increase of DI in Daraban and Qushtap regardless the low of C/N ratio may attributed to the inappropriate conditions for the transformation of nitrogen to more ammonia in soils of both areas.

The increase of disease incidence of root-knot nematodes in some areas is attributed to several factors such as suitable of temperature for nematode activity and development, this was also mentioned by Trudgill, (1995); Park *et al.*, (2005) and Isamil *et al.*, (2012) and availability of soil moisture, as well as planting of susceptible cucumber cultivars, additionally soil of the same greenhouse may be planted by susceptible hosts before autumn season and remaining debris of the infected roots in the soil which contributed in increasing infection criteria in autumn compared to spring season. However, decreasing of disease incidence in spring season is due to low temperature in the winter in addition to the absence of the host,which caused death of many nematode eggs and juveniles. This idea was affirmed by Ami (1985); Ami and Al-Sabie (1989) and Ami and Shingaly (2018). The absence or decrease of the disease incidence especially in Kamosak (Grd Goran) and Khabat is attributed also to the use of several methods for controlling nematods such as fallowing, animal manure, planting of garlic between cucumber plants or the use of nematicides.

3- Molecular diagnosis of different isolates of root – knot nematodes *Meloidogyne* spp on cucumber plant under greenhouse conditions in Erbil province:

3-1 : Electrophorized of amplified partial 5.8S rRNA gene in 1.5 % Agarose gel: By using two universal primers TW81-F and AB28 Actin – R in a PCR reaction, the bands of amplified DNA products of the Internal Transcribed Spacer (ITS) for the ten nematode isolates were clarified and visualized by agarose gel electrophoresis with lanes 1 – 10 in the same position at 500 bp (Figure,4). This indicates that both primers were effective in amplifying DNA extracted from mature females of the ten root – knot nematode isolates parasitizing on cucumber plants..This result consistents with

what AL–Sinjary (2017) found for *M. javanica* on cucumber and with **AL- Sinjary** and Ami (2022) for *M. javanica* and *M. incognita* on the same host plant in Duhok province using the same universal primer pairs. These findings indicate that it is not possible to diagnose root-knot nematode species relying only on the use of universal primer pairs and agarose gel electrophoresis therefore, it is necessary to use either species specific primers or sequencing of the amplified DNA products to accomplish the stated objective, that is.

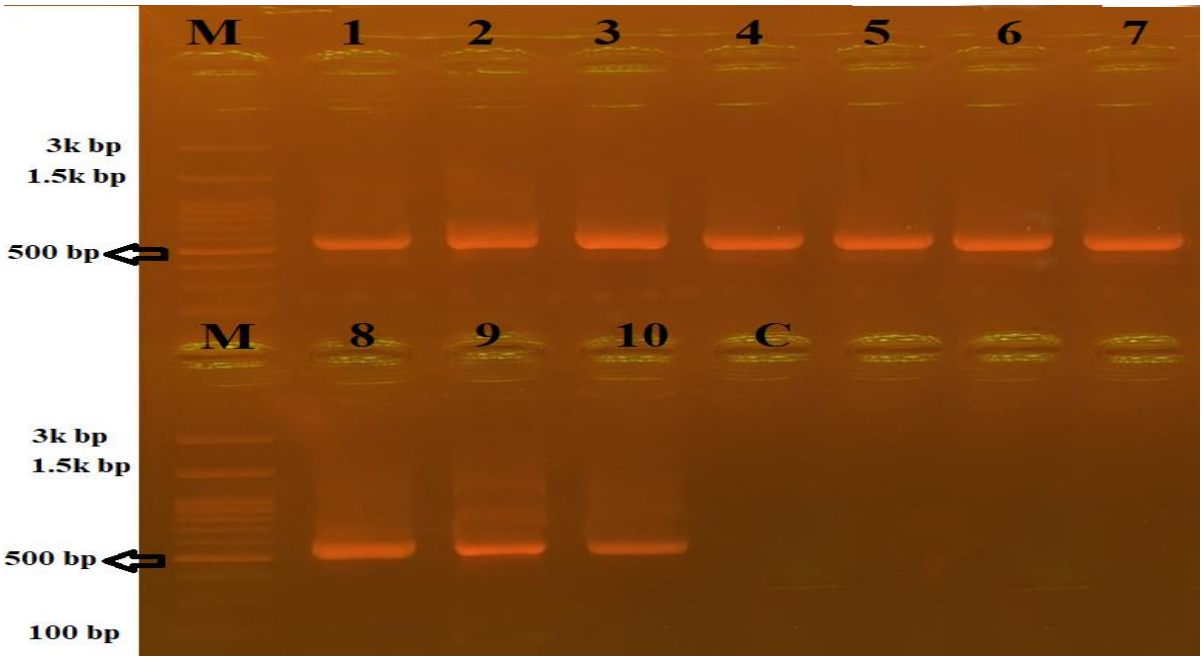

Fig. (4) : Agarose gel electrophoresis of the P C R products, M is a Marker ladder, Lane 1,2,3, 4,5,6,7, 8,9 and 10 are positive products for pair of primers (TW81-F with AB28 Actin-R) for the ten nematode isolates diagnosed in this study and C is a negative control of sterile distilled water.

to distinguish nematode species from each other. On the other hand, the sequence quality findings revealed the existence of four *Meloidogyne* species parasitizing on cucumbers distributed in surveyed locations namely *M. incognita* (Kofoid and White,1919) Chitwood, 1949 (Isolate 1Ma1.E. and 1M2.E.) and *M. javanica* (Treub,1885) Chitwood,1949 (Isolate (1Ma3.E.) in Matsawa location, with three species in Qushtapa location included *M. incognita* (Isolate 2Qu1.E.), *M. javanica* (Isolate 2Qu2.E.) and M..*arenarai* (Neal,1889) Chitwood, 1949 (isolate 2Qu3.E.). Regarding Khabat location only one species was diagnosed included *M.hapal* Chitwood, 1949 (Isolate 3 Kh. E.) As well as three species were identified in Draban location included M..*arenarai* (isolate 4 Da1. E.). *M.javanica* (isolate 4 Da2. E.) and *M.hapla* (isolate 4 Da 3. E.), Thus, four main species were diagnosed due to identifying some nucleotide variations between them. The NCBI subsequently
determined the accession number for each species and isolate as shown in table (4).

Table (4) :- Species and isolates of root – knot nematodes *Meloidogyne* spp. with their accession number that have been diagnosed on cucumber plants in surveyed locations in Erbil province

Sample number	Nematode isolate	Accession Number	Nematode species	Location
1	1Ma1.E.	**ON833423.1**	*M. incognita*	Mastawa
2	1Ma2.E.	**ON833424.1**	*M. incognita*	
3	1Ma3.E.	**ON833425**	*M. javanica*	
4	2Qu1.E.	**ON833426.1**	*M. incognita*	Quahtapa
5	2Qu2.E.	**ON833427.1**	*M .javanica*	
6	2Qu3.E.	**ON833428.1**	*M. arenaria*	
7	3 Kh. E.	**ON833429.1**	*M. hapla*	Khabat
8	4 Da1. E.	**ON833430.1**	*M. arenaria*	Daraban
9	4 Da2. E	**OQ693824**	*M. javanica*	
10	4 Da3. E	**OQ693825**	*M. hapla*	
Host plant	Cucumber (*Cucumis sativus* L.)			

Ma = mastawa. Qu = Qushtapa. Kh = Khabat. Da = Daraban.

2- Genetic similarity of root – knot nematode isolates : Results of genetic similarity as clarified by the blast program's description of the Query ID. for comparing nematode species and isolates diagnosed in this study with each other showed that only two isolates of *.M.incognita* were found to have 100% similarity

that were isolate 1Ma 2.E and 2Qu1.E.from Mastawa and Qushtapa locations, respectively, which indicate that both isolates belong to the same race, while the other isolates varied genetically to clarify the existence of two races for each of *M. incognita* (Isolate 1Ma1.E vs both isolates 1Ma2.E and 2Qu1.E), *M.javanica* (Isolate 1Ma3.E vs isolate 2QU2.E.) and *M.arenaria* (Isolate 2QU.3.E. vs isolate 4 Da1.E.) where genetic variation between races in each case reached 3.73, 0.22 and 0.67% ,respectively. In general the highest percent identity (99.78 %) among isolates that differed genetically was recorded between *M. javanica* isolate1Ma3.E from Mastawa location and the same species isolate 2Qu2.E from Qushtapa location (Table,5) as emphasized also by the result of a DNA Dot Plot for both isolates (Figure, 5),while the lowest percent identity (84.9%) was observed between *M.incognita* isolate 1Ma2.E from Mastawa location and *M.hapla* isolate 3Kh.E from Khabat location (Table,5) which was confirmed also by the result of a DNA Dot Plot of both species (Figure, 6).

Table (5) : The Percent Identitiy between the ten root – knot nematode species and their isolates diagnosed in this study with each other based on the blast of Genbank NCBI of partial 5.8S rRNA..

Root – knot nematode species and Isolate	*M .i* 1Ma 2.E .	*.M. j* 1Ma 3.E.	*M.. i* 2Qu1.E.	*M. .j* 2Qu 2.E.	*M.. a* 2Qu 3.E .	*M. h* 3Kh. E.	*M. a* 4 Da 1. E.	*M. j* 4 Da 2. E.	*M. h* 4 Da 3. E.	Location
M. I 1Ma 1.E. ON833423.1	96.27	99.1	96.27	99.12	99.48	88.78	99.18			Mastawa
M. I 1Ma 2.E. ON833424.1	100	96.34	100	96.14	96.86	84.90	96.54			
M. j 1Ma 3.E . ON833425.1	96.34	100	96.33	99.78	99.28	86.82	96.54			
M. I 2Qu1.E . ON833426.1	100	96.33	100	96.14	96.86	84.90	96.53			Quahtapa
M .j 2Qu 2.E. ON833427.1	96.14	99.78	96.14	100	99.29	86.51	99.35			
M. a 2Qu 3.E . ON833428.1	96.86	99.28	96.86	99.29	100	87.93	99.33			
M. h 3Kh. E . ON833429.1	84.90	86.82	84.90	86.51	87.93	100	86.50			Khabat
M. a 4 Da1. E. ON833430.1	96.54	96.54	96.53	99.35	99.33	86.50	100			Daraban
M. j 4 Da2. E. OQ693824.1	No significant similarity found							100	88.64	
M. h 4 Da3. E . OQ693825.1	No significant similarity found							88.64	100	

M .i = M.incognita. M .j = M.javanica. M. a = M. arenarai . M .h = M. hapla. Ma = mastawa.
Qu = Qushapa. Kh = Khabat. Da = Daraban

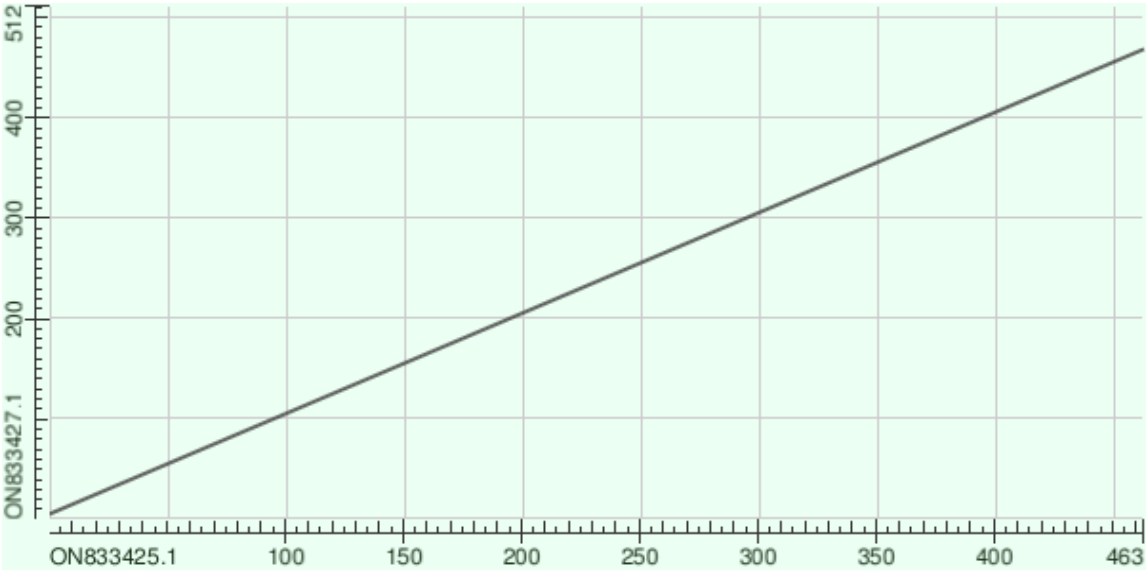

Fig. (5) :- A DNA Plot of *M .javanica* isolate1Ma3.E. (ON833425.1) from Mastawa location vs the same species isolate 2Qu2.E. (ON833427.1) from Qushtapa location

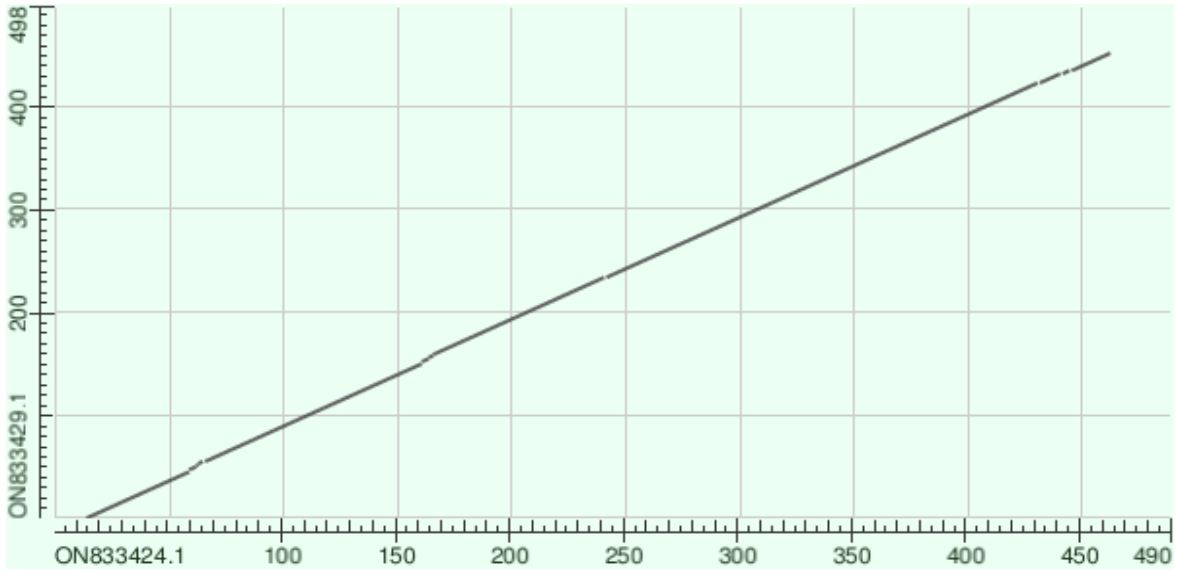

Fig. (6) : --A DNA Plot of *M .incognita* isolate1Ma2.E (ON833424.1) from Mastawa location vs *M. hapla* isolate 3Kh.E. (ON833429.1) from Khabat location.

This results confirm results of the previous studies in Iraq on the presence of two races of each of *M. incognita* and *M.arenaria* (Al-Sabie and Ami,1990) and two races of *M. javanica* with one race of *M.hapla* on different host plants (Stephan, 1997). However, this study is the first record of these races according to the genetic differences between them. No significant similarity was found between each of *M. javanica isolate* 4 Da2. E .and *M.hapla* isolate 4 Da3.E.from Daraban location and the other species and isolates which may suggest that they belong to two different races.

Regarding the genetic similarity of species and isolates diagnosed in this study compared to the other species and isolates of root – knot nematodes diagnosed previously in Kurdistan Region – Iraq and deposited in NCBI genbank, it was found that the percent identity was less than 100% which means that the same species and isolates diagnosed in this study belong to different races compared to the same species diagnosed previously in Kurdistan Region – Iraq according to the genetic variation between them. In general the highest percent identity (99.53%) was found between *M .javanica* isolate 1Ma3.E.(ON833435.1) from Mastawa location and the same species isolate MJ-32 (MW168165.1) from Duhok province (Table,6). as confirmed by the result of a DNA Dot Plot of

both isolates (Figure,7), Meanwhile the lowest percent identity (85.09%) was showed between *M.hapla* isolate 3Kh.E.(ON833429.1) from Khabat location and both isolates of *M.incognita* (A-1 ON677754.1 and B-2 ON677754.1) from Duhok province as emphasized by the result of a DNA Dot Plot of both species (Figure,8). No significant similarity was found between each of *M.javanica* isolate 4D2.E (OQ693824.1) and *M.hapla* isolate 4D3.E (OQ693825.1) collected from Daraban location and the other species and isolates either diagnosed in this study or previously in Kurdistan Region – Iraq (Table,6).

Table (6):-Percent Identity between the ten root – knot nematode species and their isolates diagnosed in this study compared to the other species and isolates of the same nematode genus diagnosed previously in Kurdistan Region - Iraq according to the blast of Genbank NCBI of partial 5.8S rRNA

Root – knot nematode and isolates diagnosed in this study	**Root – knot nematode species and isolates parasitized on cucumber and previously diagnosed in Kurdistan Region – Iraq**				
	M. arenaria isolate E 3 (ON677755.1)	*M.incognita* isolate A-1 (ON677753.1) and B-2 (ON677754.1)	*M. incognita* isolate CN36 (MW152160.1)	*M. javanica* 4 similar isolates *	*M. javanica* isolate MJ -32 (MW168165.1)
M. incognita. 1Ma 1.E. ON833423.1	99.22	97.29	98.31	98.60	98.45
M. incognita. 1Ma 2.E. ON833424.1	96.28	96.53	97.99	95.87	95.82
M. javanica . 1Ma 3.E. ON833425.1	99.14	98.49	99.19	99.14	99.53
M. incognita. 2Qu1.E. ON833426.1	96.27	96.52	97.99	95.86	95.81
M .javanica. 2Qu2.E. ON833427.1	98.96	98.34	98.94	99.01	99.30
M. arenaria. 2Qu3.E. ON833428.1	99.11	97.71	98.57	99.27	98.39
M. hapla. 3 Kh. E . ON833429.1	86.21	85.09	86.64	86.37	85.60
M. arenaria 4 Da1. E.. ON833430.1	98.75	98.12	99.24	98.75	99.07
M. javanica 4 Da2. E.. OQ693824.1	No significant similarity found				
M. hapla 4 Da3. E.. OQ693825.1	No significant similarity found				

*This species of includes four isolate namely, C-3 (ON667903.1), D-4 (ON667904.1), F-6 (ON667905) and 1-7(ON667906) .

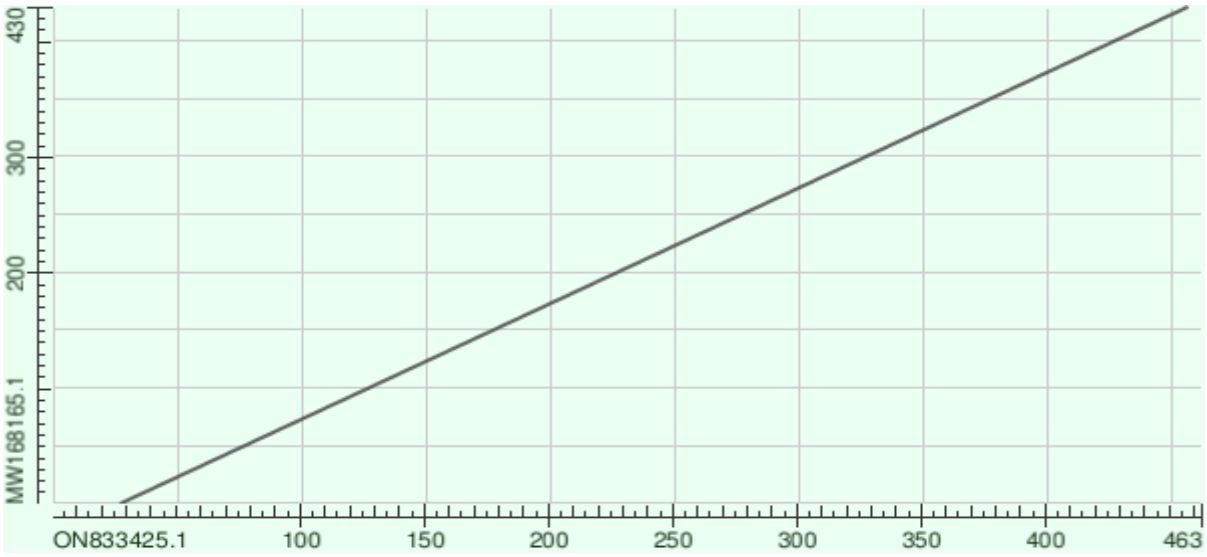

Fig. (7) : -A DNA Dot Plot of *M .javanica* isolate1Ma3.E. (ON833425.1) from Mastawa location vs the same species isolate MJ-32 (MW168165.1) from Duhok province.

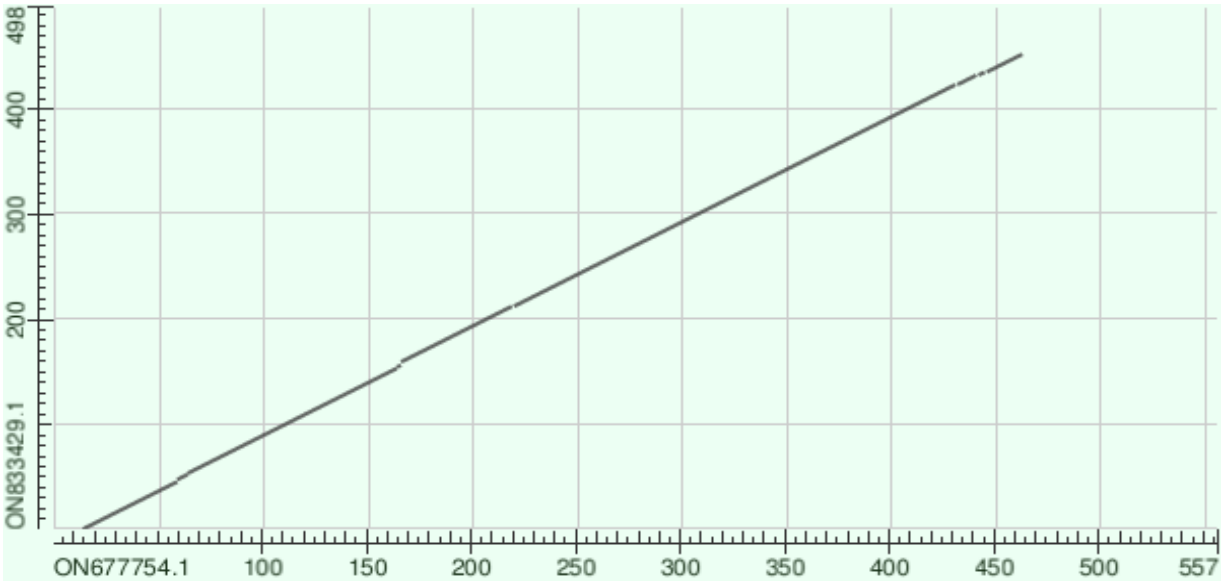

Fig. (8) : -A DNA Plot of both isolates of *M .incognita* A-1 (ON677753.1 and B- (ON677754.1) from Duhok province vs *M. hapla* isolate 3Kh.E.(ON833429.1) from Khabat location.

CONCLUSIONS

Results indicated that the highest disease incidence (D.I.) (87.5%) was recorded during autumn 2021 on cucumber plants in the greenhouses of Qustapa / Erbil province, whilst no infection (D.I. 0.0%) was recorded on cucumber in Kamosak (Grd Goran) location in both growing seasons and in Mastawa location during spring season 2022. Using molecular diagnosis four root- knot nematodes species parasitizing on cucumber were recorded namely. *M. incognita, M. javanica, M. arenaria and M. hapla* and according the genetic variation two races for each of the first three species were recorded for the first time by this technique.

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