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First report and molecular confirmation of the potential entomopathogenic nematode Oscheius tipulae in Baghdad, Iraq

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Abstract: *A survey was conducted from five locations with three different field sites in Baghdad ,Iraq. The nematodes were recovered from soil samples and isolated from Galleria mellonella larvae as baits. The nematode isolate was found in the region of Al-Usefya(Alfalfa). Nucleotide sequence analysis of partial (cox1) gene confirmed Oscheius tipulae identification when shared maximum 97% nucleotide identity with equivalent sequence from NCBI. Neighbor tree showed high relatedness of O.tipulae with British isolate CP0590341 suggesting common origin. The Oscheius tipulae isolate was the first record of one local species in Iraq.*

Keywords: *Survey , Entomopathogenic nematodes, Oscheius tipulae Molecular identification, Iraq*

INTRODUCTION

Entomopathogenic nematodes (EPNs) todate are used as bio – control agents of insect pests in agricultural fields and home gardens.(Ahmed and Ali، 2009) .The EPNs characterization is particularly useful to match the appropriate EPN species with a targeted pests and environmental conditions for an effective bio control potential and integrated pest management programs (IPM) (Peat etal،2009) (AL-Obaidy ،2019).. Polymerase chain reaction and DNA sequencing techniques have played an important role in advancing the EPN taxonomy ,biodiversity , geographic distribution , host ranges ,ecology behavior and co-evolution . (Lulamba and Serepa-Dlamini 2020). Molecular characters are considered as the most suitable approach for species identification of nematodes and the morphologically similar species (Campos – Herrera، 2015) (Al-Khazraji etal، 2016).

The aim of this study was to isolate and identify native EPNs species that can be suitable as a bio- control agent by testing it against *Galleria mellonella L.* under laboratory conditions.

MATERIALS AND METHODS

Soil Sampling and Isolation

Soils were collected from five locations with three different field sites including Al-Jadrya (Alfalfa, Eggplant , Citrus) and Abu-Ghraib (Alfalfa, Wheat , Okra) and Al-Rashdiya (Citrus ,Green pepper , Clover) and Al-Usefiya (Broad beans ,Alfalfa ,Okra) and Al-Madayin (Alfalfa , Apricot, cucumber) in Badghdad ,Iraq.(Al-Saidi and Al-Obaidy ،2022).(Horamani and Saramany، 2024).

Soil samples were collected , 10 – 15 cm below the soil surface . The insect – baiting technique of (Bedding and Akhurst 1975) using the last instar larve of the greater wax moth, Galleria mellonella L., the soil samples were transferred into plastic containers 250 ml with lids. 5-7 last instar larve were used for each container. These containers with the larve were placed under lab

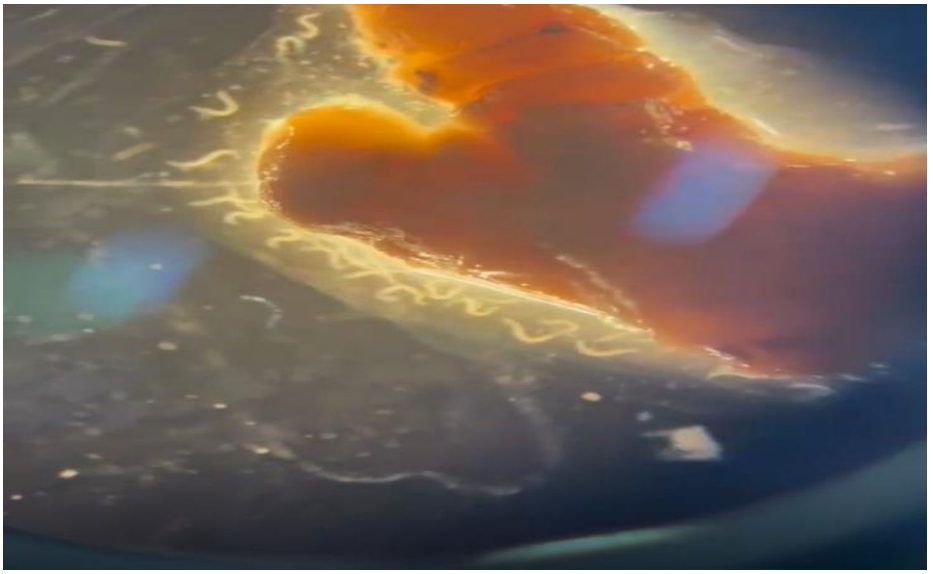
conditions for 5-7 days. To encourage movement of nematodes in the soil , the containers were checked everyday to remove infected larve with EPNs. The infected larve were transferred to white traps individually (white 1927). The samples were stored in the incubator (24-28) C°, after 9 - 10 days, infected juveniles IJs emerged from the cadavers. (Kaya and Stock 1997). To sum up , the emerged IJs were maintained and stored inside a tissue flask and took place in refrigerator under (8 – 10) C° for next step.

MOLECULAR CHARACTERIZATION OF PNS

DNA of nematodes was extracted using kit provided by Geneaid Co. (Korea). Nematode suspension samples were collected and transferred into a 1.5 ml micro tube and centrifuged at 13500 cycle / min . 200µl Buffer GST and 30µl proteinase k^2 were added , the samples were placed in water bath 60C° for 1 hour and shacked then centrifuged at 1400 – 1600 cycle/ 2 min. GSB buffer 200µl and 200µl Ethanol absolute were added to the supernatant. The supernatant was placed through GS columns. 400µl W1 Buffer and 600µl wash Buffer was added. . Program was used as follows: initial denaturation 95C° 5 min 1 cycle, 95C° 30 sec 35 cycles , 45C° 30 sec , 72C° 1 min , 72 C° 5 min. The amplified fragments were separated through electrophoresis on a 2% Agarose gel in 100 ml TBE buffer (1x) . Bioneer Co. (Korea). PCR products were sent to Macrogen Co. (Korea) .(Yaser and Al-maliky،2024).

RESULTS AND DISCUSSION

The nematodes were recovered from soil samples and isolated from *Galleria mellonella* larvae as baits according to the procedures described by (Bedding and Akhurst 1975). The nematode isolate was found in the region of Al-Usefya (Alfalfa) identified as *Oscheius tipulae*. It is the first record of the nematode local isolate infecting *Galleria mellonella* larvae in Baghdad , Iraq with accession number PQ432530. (AL-Obaidy etal،2019). Figure (1) and (2) showing infected larvae of *Galleria mellonella* by nematodes Oscheius tipulae.



Figure(1): showing infected Galleria mellonella larvae in microscope.

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Figure (2): showing infected Galleria mellonella larvae in petri dish.

Nucleotide sequence analysis of partial (cox1) gene confirmed *Oscheius tipulae* identification when shared maximum 97% nucleotide identity with equivalent sequence from NCBI figure (2).

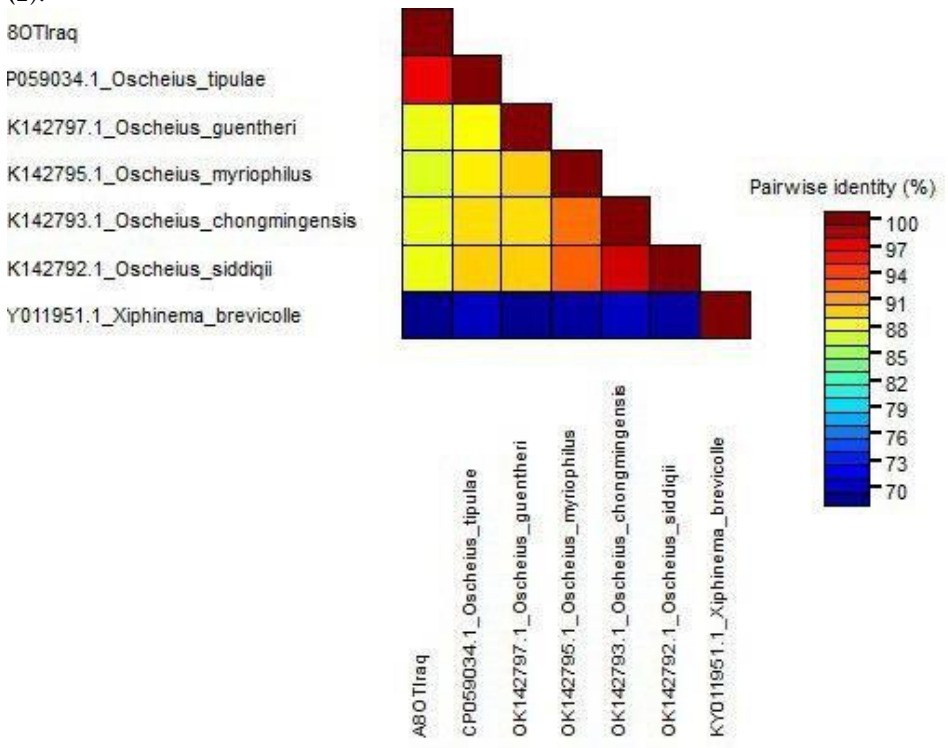

figure (3): Nucleotide sequence identities of partial cytochrome oxidase subunit 1 (cox1) gene amplified from the nematode *Oscheius tipulae* isolated in this study (referred to as A8OTIraq) and equivalent isolates/species from NCBI. The nematode *Xiphinema brevicolle* was included as an out-group comparison. A colored matrix was generated using SDTv 1.3 software (Muhire et al. 2014).

Tree showed high relatedness *Oscheius tipulae* with British isolate CP059034.1 suggesting common origion. figure (3).

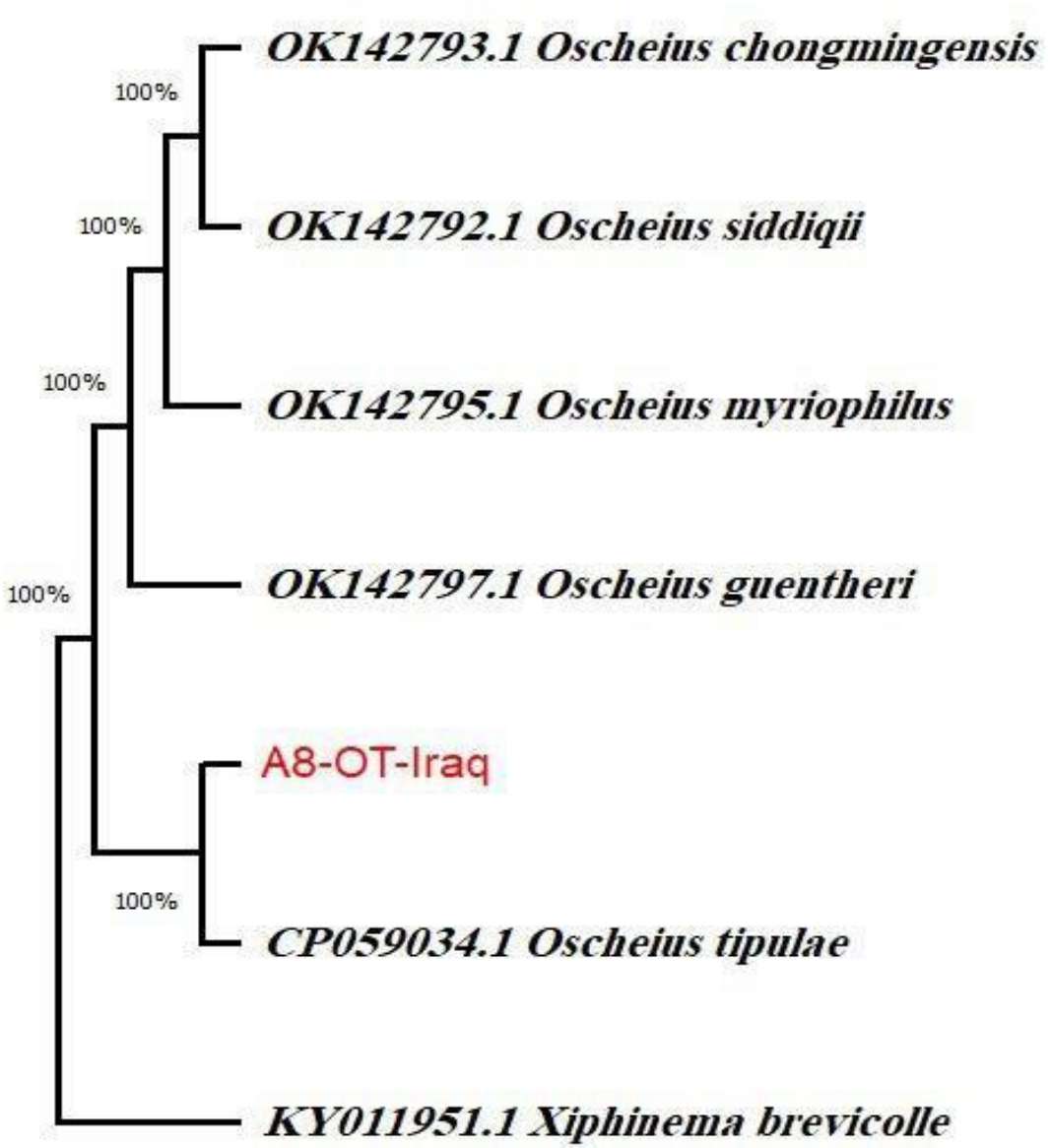

Figure (4): Neighbor-Joining phylogenetic tree of the nematode *Oscheius tipulae* isolated in this study (referred to as A8OTIraq) and equivalent isolate/species from NCBI. The nematode *Xiphinema brevicolle* was included as an out-group comparison. A colored matrix was generated using MEGA 11 software (Tamura et al. 2021).

CONCLUSION

This study documented the first record of one local species identified as *Oscheius tipulae* isolated from *Galleria mellonella* in middle part of Iraq. The presence of *O.tipulae* isolate in a dry hot climate in Iraq is a significant contribution to the biogeography of these species and the use of EPNs has been regarded as one of the highly promising biological control strategies due to their capacity to efficiently control different insect pests with a well-developed mass production technique.

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