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**POPULATION DYNAMICS OF DAGGER NEMATODE ATTACKING  
ALEPPO PINE TREE IN JORDAN**

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**ABSTRACT**

The temporal distribution of an isolate of the dagger nematode attacking Aleppo pine, *Pinus halepensis* grown in AL-Jubiha area in Jordan was investigated. A total of eighteen samples were collected from rhizosphere about 30 cm deep in soil and 50 cm away from the trunk of a Pine tree showing decline and brown needles as one sample per month starting in November 2014 until April 2016. Cobb sieving and gravity methods were used for the nematode isolation from rhizosoil. The soil type is clay with 51 % porosity. The monthly air temperature, precipitation, and relative humidity were monitored and tabulated. The results showed that the number of recovered nematodes ranged from 2 individuals /100 cm<sup>3</sup> to 88 individuals /100cm<sup>3</sup> of rhizosoil. The lowest number was recovered on October whereas the highest numbers were recovered in December. The reason of decline in numbers may be due to a raise in temperature. The highest number may be due to favorable temperature and soil moisture. The difference of nematode in same month in two different years may be due to the temperature and precipitations.

**Keywords:** Aleppo pine, dagger nematode, population, temperature, moisture.

**INTRODUCTION**

Forest trees are basic components of an ecosystem in Jordan which occupies now less than 1% of Jordan total area dominated by Aleppo pine, *Pinus halepensis*. It is severely affected by several factors and thus it is critically endangered (Al-Eisawi, 2012). Each year, large areas of Aleppo pines forests are destroyed by outbreaks of pathogenic fungi, viruses, insects, nematodes, and recently phytoplasma (Seliskar, 1966; Lieutier and Ghaioule, 2006; Karadžić and Vujanović, 2009; Botella *et. al.*, 2010). Only one species belonging to the spiral nematode, *Helicotylenchus digonicus* and two species belonging to the dagger nematodes, *Xiphinema pachtaicum* and *X. vuittenezi*, were recorded to be associated with roots of *P. halepensis*, in Jordan (Hashim, 1979; EPPO quarantine pest, 2006). Dagger

nematodes, belong to the genus *Xiphinema*, are migratory ectoparasites and several species belong to this genus were recorded to be associated with pine trees worldwide (Griffiths *et. al.*, 1982). Riffle (1972) recorded indirect effect of some *X. americanum* on pine trees since it directly attacked ectomycorrhizae associated with seedlings of *P. edulis* leading to decline in pine growth.

Recently, it has been noticed that several trees of Aleppo pine grown in the campus at the University of Jordan at Jubeiha are weak and several needles are brown in color (Luma Al Banna observations). The dagger nematode was found in the rhizosoil of the roots of those weak trees. This study aimed to identify the species of this nematode and furthermore to investigate the temporal distribution of this dagger nematode attacking Aleppo pine.

## MATERIALS AND METHODS

**Sampling and Extraction of Dagger Nematode Isolate:** Soil samples from rhizosphere of a 25m tall Aleppo pine *tree* grown in Jordan university campus were collected monthly. The tree appeared weak with many dead branches, the remain green branches also have several brown colored leaves while the surrounding trees look healthier than the sampled tree. Sampling started in November 2014 until April 2016, with a total of 18 samples. The samples were collected from the rhizosoil about 50 cm away from the trunk base of the tree. The upper 30 cm soil were excluded and rhizosoil sample were collected with a shovel from the next 30-40 cm deep of soil. The rhizosoil cores were stored in small plastic bags, and properly labeled. Air temperature, precipitation, and relative humidity were monitored monthly and the data were tabulated. Rhizosoil samples were stored in cold temperature at 4-8 C until used. Cobb sieving and gravity method was followed as described by Hooper (1986) to extract the dagger nematode from the 18 samples.

**Soil Analysis:** Physical and chemical properties of rhizosoil samples which was collected in December 2015 from tested Aleppo pine tree were determined. Such properties include: soil moisture content, soil particle density, soil bulk density, porosity, organic matter percentage, soil pH, electrical conductivity, soil texture and soil fraction.

**Effect of Cold Storage of Rhizosoil Samples on the Number of Dagger Nematodes:** To investigate the effect of cold storage on dagger nematode population, a monthly specimen was taken from a rhizosoil sample which was collected in 9 March 2015, and stored at 4 - 8 C. The extraction repeated once monthly for six months of cold storage. Nematode extraction was performed as mentioned earlier.

**Identification of Collected Nematode Sample:** Recovered nematodes were examined and counted using a dissecting microscope. Mounts of the recovered nematodes were prepared for the purpose of identification. Temporary and permanent mounts of the recovered nematodes were prepared following in order Seinhorst slow method (Seinhorst, 1959). Mounts were examined using a light compound microscope. Both qualitative and quantitative morphological characters

were documented. These morphological characters were used to identify the species of the dagger nematode following original descriptions and diagnostic keys (Luc *et. al.*, 1964; Groza *et. al.*, 2013).

**Temporal Distribution of the Recovered Dagger Nematode:** Monthly total numbers of recovered nematodes from 100cm<sup>3</sup> of rhizosoil (from November 2014 until April 2016) were tabulated and a histogram was established.

### RESULTS AND DISCUSSION

The analyses of the collected rhizosoil samples revealed that the soil type is a clay soil with 17.5% sand, 30 % silt and 52.5% clay. Clay soil is compact soil that is not preferred to harbor dagger nematodes due to low porosity and reduction level of oxygen, however, the presence of organic matter (7.2 %) which surrounds the soil particles with small particle density 2.05 g/cm<sup>3</sup> resulted in high pore space (51%) and lower bulk density (1 g/cm<sup>3</sup>). Similarly, Harris (1979) reported that under conditions of limited moisture, nematode reproduction increases in fine-textured soil with a greater moisture holding capacity. The pH of the sampled rhizosoil was 7.84 which is considered suitable for the survival of dagger nematode. The recovered dagger nematode was identified as *Xiphinema vuittinezi*. All specimens were longer than the previously recorded populations that associated with pome fruits, grapevine and stone fruits in different area in Europe, USA, Australia, Czech Republic and Iran (Groza *et. al.*, 2013). This variation can be due to differences in host plant, geographical location, and other environmental conditions.

**Temporal Distribution of the Recovered Dagger Nematode:** Seasonal variation of *X. vuittinezi* around roots of Aleppo pine at Jubiha from 17 November 2014 till 28 April 2016 as total number of individuals /100 cm<sup>3</sup> of rhizosoil are shown in Figure (1). Monthly air temperatures and precipitations are also shown in Figure (1).

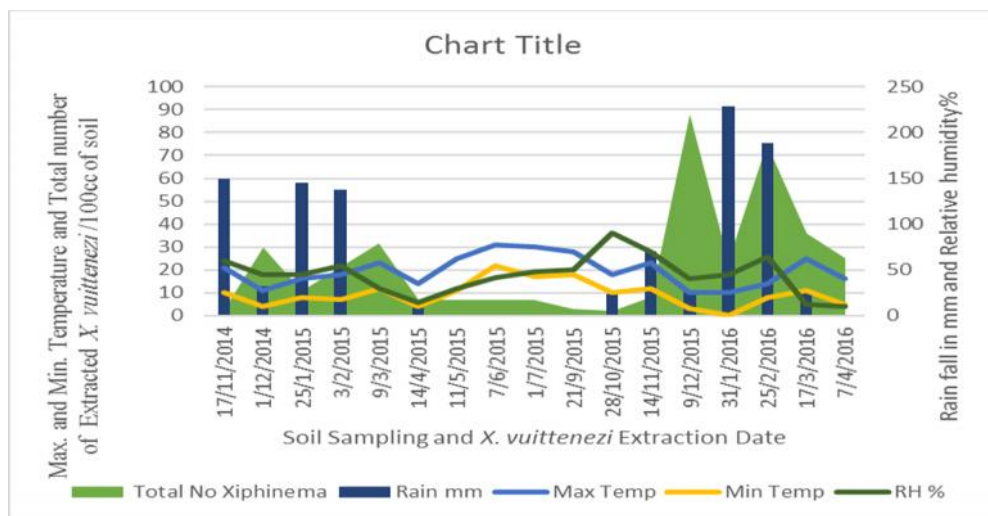


Figure 1. Seasonal population fluctuations of *Xiphinema vuittenezi* around roots of Aleppo pine at Jubiha, Amman= Jordan from November 2014 till April 2016.

The results showed that the number of recovered nematodes ranged from 2 individuals /100 cm<sup>3</sup> to 88 individuals /100cm<sup>3</sup> of rhizosoil. The lowest number was recovered in October 2015 whereas the highest numbers were recovered in December 2015. The economic threshold (ETH) for dagger nematode was determined to be 50–100 individuals /100cm<sup>3</sup> of soil for horticultural crops (Ravichandra, 2014). This can explain the observed damage of sampled tree since the highest recovered population during 2015 reached 88 individuals /100 cm<sup>3</sup> of soil while it was only 30 individuals/100cm<sup>3</sup> of rhizosoil in previous year. This can explain the slow effect of this nematode on pine trees which increase year after year. There was low number of *X. vuittenezi* during November 2014 but after rain fall started, the population increase in next December, Similarly, when the rain falls started again during October 2015 a remarkable population increase was recorded in November 2015. The low number of recovered nematodes from April to October 2015, might be due to low soil moisture since no precipitation events were recorded during this period. This result is similar to Feil, *et. al.*, (1997) who recorded that period of soil dryness may increase reproduction rate of *X. index* and increase population level during next winter season. Low temperature averaged to 16 C from November 2014 till March 2015 with presence of high soil moisture elevated the recovered numbers of *X. vuittenezi*. However, during January 2015 the population declined sharply from 30 individuals /100 cm<sup>3</sup> in December 2014 to 12 individuals /100 cm<sup>3</sup> and similar trend was also recorded next year where the population declined from 88 individuals /100 cm<sup>3</sup> in December 2015 to 24 individuals /100 cm<sup>3</sup> in January 2016 which may be attributed to the very low temperature as a result of snowing event during this month. Sharp decline in population was recorded from May (7 individuals /100cm<sup>3</sup> of rhizosoil) to the end of October (2 individuals /100cm<sup>3</sup> of rhizosoil) as a result of reduced soil moisture due to elevated air temperature above 20 C. this is in similar to Griffin *et. al.*, (1996) who reported that the optimum temperature for *X. americanum* was 21 C, and the nematode cannot survive high soil temperatures but it can survive winter months at low temperatures. Both soil moisture and temperature have an effect on population of *X. vuittenezi* and this is in similar to Ferris and Mckenry (1974) who found that soil moisture is a critical factor for egg hatching of *X. americanum* at time of suitable temperature. The combination of suitable host roots, favorable temperature and sufficient soil moisture resulted in population build up as recorded during the months February, March and April 2016.

#### **Effect of Cold Storage on *X. vuittenezi* Population**

Soil sample during March 2015 was kept at 4-8 C for further extraction. The monthly recovered *X. vuittenezi* individuals are graphed in figure (2).

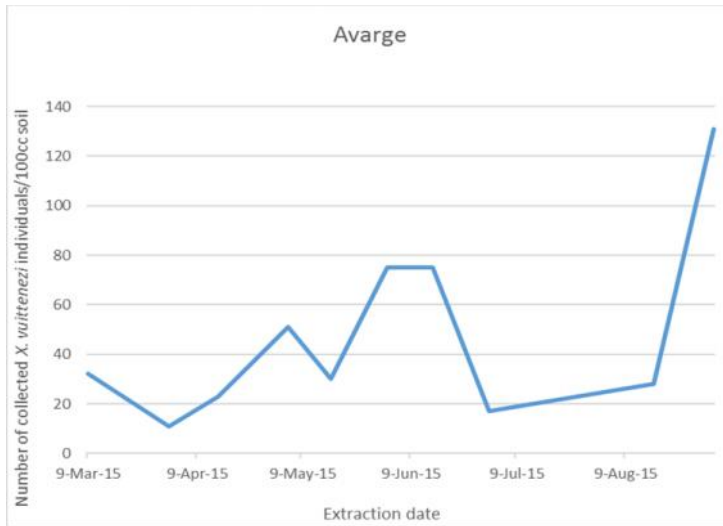


Figure 2: *Xiphinema vuittenezi* population at different extraction time from collected sample in 9 March 2015 around roots of Aleppo pine at Jubiha, Amman, Jordan

The recovered individuals declined after one month of storage. With extending the period of storage, the population fluctuated with a sharp three peaks after second, third, and sixth months of storage. Most of the recovered individuals were immature stages. The increase of the nematode number might be because of egg hatching or the end of a quiescent stage.

### CONCLUSION

This study illustrates the presence of high population of *X. vuittenezi* within the soil of studded Aleppo pine tree. The population dynamic of this nematode show fluctuation during the sampling period as a reflect of several biotic and abiotic factors. Cold storage of a soil sample also resulted in fluctuation of collected *X. vuittenezi* individuals. More biological and ecological studies are needed to understand the critical damage of this nematode on forest Aleppo pine trees in Jordan.

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