

Determine the Distribution of Nematode Infestation Bud and Leaf Nematode of Strawberry Plants in Mazandaran Province, North of Iran

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ABSTRACT: During the years 2012 to 2014, Eighty three soil and foliage samples were collected from different parts of the strawberry fields including Joubar, Kiakola, Vazimal, Rohnkola, Jamalkola, Bahnamir, Eznava, and Behshahr. *Aphelenchoides* spp. was isolated from 24 foliage samples. The population density of nematodes in 50 g samples ranged from 10 to 2000, while only 10 soil samples from these shoots were infested with *Aphelenchoides* spp. The population densities of nematodes were determined from 30 to 25,000 per 50 grams of shoots and 20 to 12,000 per 250 cm³ of soil. *Aphelenchoides* species identified in this study consisted of *A. fragariae*, *A. nechaleos* and *A. macromucron* which described in discussion part. The three identified *Aphelenchoides* species are recorded for the first time from Iran.

KEY WORDS: *Aphelenchoides* species, Nematode, Strawberry

I. INTRODUCTION

Strawberry cultivation is about ten thousand hectares in Iran. Kurdestane province with 2500 thousand of strawberry cultivation has the largest amount of acreage in Iran. Cultivation in Mazandaran, is about one thousand and hundred hectares [1]. Kumaso and Silva are the main varieties planted in different cities of the province. Strawberry plant that grows in the province as well as other products has a great range of nematodes that also cause damage. Enelyand Aztork [2] in Turkey reported the leaf nematode of the most important nematodes are damaging. Previously reported, there are some *A. besseyi* nematode existed in the strawberry field of joybar in Mazandaran province [3]. Usually species *A. fragariae*, *A. besseyi*, *A. ritzemabosi* and *D. dipsaci* out damage to strawberries [4]. Hassan et al. in turkey [5] reported *Aphelenchoides fragariae* for Strawberry. Park et al [6] examined plant parasitic nematodes refer strawberry in South Korea and reported about 11 species of plant parasitic nematodes; *M. arenaria*, *A. fragariae*, *Meloidogyne incognita* and *Paratylenchus penetrans* are the important species which has economic value. Chizhov et al [7] reported *A. fragariae* nematode as a species which produced disease in strawberry of Moscow.

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II. MATERIAL AND METHOD

During the 2013 till 2015 of 83 samples from the aboveground strawberries and soil around the roots of this product were collected in the Mazandaran province. 50 grams of leaves and stems were mixed and studied. Farm was from half to two hectare. By mixture of 5 samples, we gain 50 grams of leaves and stems were investigated. Examination of samples was done by Coolen and D, Herde method [8]. In order to extract nematodes from soil, 250 cm of the soil samples was examined using sieves and centrifuge [9]. Extracted nematodes were identified and counted under the microscope. In order to identify nematodes were fixed and transferred to glycerin. Identification of nematodes was conducted according to morphology and morphometric characteristics. In two species identification of molecules, was done by using DNA which elicited from 6-7 nematodes of each species, the region of Ribosomal gene of D2-3D 285 duplicate with primer of D3B and D2A [10]. Then the product of polymerase chain reaction was sent to the Bioner Company in South Korea for Nucleoid order determination. The resulting sequence alignment and comparison of sequences in the gene bank of the world.

III. RESULTS

The results of samples are shown in Table 1. Out of 83 samples of aboveground were studied in 27 samples of *Aphelenchoides* sp. with a population of 10 to 2000 nematodes were found in 50 grams of tissue. While in the 10 soil samples from the same shoot nematode *Aphelenchoides* sp. were separated. Identified *Aphelenchoides* species in this examination consisted of *A. fragariae*, *A. nechaleos*, *A. macromucrons*, which explain in discussion section. All three species of identified *Aphelenchoides* is first time that reported from strawberry plant of Iran. Among the identified species, *A. macromucrons* were separated from large number of region.

Table 1. Samples of strawberry plantations in the Mazandaran province with isolated nematodes and their populations

Type of nematode	Source Collection		Number of samples	Sampling place and name of owner of the farm	rank
	Leaf(5gr)	Soil(250gr))			
	population	population			
<i>Aphelenchoides</i> sp	160	0	4	Behshahr-khalilshar(Saro)	1
<i>Aphelenchoides</i> sp	20	0	3	Kiakola- Vazimal (Mohseni)	2
<i>Aphelenchoides</i> sp	50	50	1	Kiakola-Vazimal(Azizi)	3
<i>Aphelenchoides</i> sp	0	0	1	Roknkola(Esmaeli)	4
<i>Aphelenchoides</i> sp	10	0	1	Kiakola-Vazimal(Azizi)	5
<i>Aphelenchoides</i> sp	25	0	2	Roknkola(Ebrahimi)	6
<i>Aphelenchoides</i> sp	20	150	1	Kiakola-Vazimal(Azizi)	7
<i>Aphelenchoides</i> sp	50	40	2	Kiakola-Vazimal(Azizi)	8
<i>A. macromucron</i>	20	450	2	Kiakola-Vazimal(Mohseni)	9
<i>Aphelenchoides</i> sp	30	0	2	Kiakola-Vazimal(Azizi)	10
<i>Aphelenchoides</i> sp	0	0	2	Kiakola-Vazimal(Mohseni)	11
<i>Aphelenchoides</i> sp	30	0	1	Roknkola(Ebrahimi)	12
<i>Aphelenchoides</i> sp	20	0	2	Roknkola(Ebrahimi)	13
<i>Aphelenchoides</i> sp	0	0	3	Kiakola-Vazimal(Mohseni)	14
<i>Aphelenchoides</i> sp	0	0	2	Roknkola(Ebrahimi)	15
<i>Aphelenchoides</i> sp	20	0	2	Kiakola- (Azizi)	16
<i>Aphelenchoides</i> sp	0	0	3	Vazimal(Ebrahimi)	17
<i>Aphelenchoides</i> sp	0	0	3	Joibar Roknkola(Ebrahimi)	18
<i>Aphelenchoides</i> sp	30	0	1	Kiakola-Koikhil (Mohseni)	19

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<i>Aphelenchoidessp</i>	10	0	1	(Kiakola-Koikhil (Mohseni)	20
<i>Aphelenchoidessp</i>	0	0	2	Kiakola- (Azizi)	21
<i>Aphelenchoidessp</i>	0	0	2	Kiakola(Talaei)	22
<i>Aphelenchoidessp</i>	10	0	2	Aznova(Adelian)	23
<i>Aphelenchoidessp</i>	50	0	2	Bahnameir(Jahani)	24
<i>Aphelenchoidessp</i>	20	0	2	Aznova(Adelian)	25
<i>Aphelenchoidessp</i>	140	60	2	Aznova(Adelian)	26
<i>A. nechaleos</i>		100	3	Bahnameir(Jahani)	27
<i>A. macromucron</i>	2000	10800	2	Aznova(Adelian)	28
<i>A. nechaleos</i>	20	10	3	Bahnameir(Jahani)	29
<i>Aphelenchoidessp</i>	150	100	1	Bahnameir(Jahani)	30
<i>A. fragariae</i>	40	10	3	Bahnameir(Jahani)	31
<i>Aphelenchoidessp</i>	0	0	3	Bahnameir(Adelian)	32
<i>A. macromucron</i>	500	0	2	Bahnameir(Jahani)	33
<i>A. fragariae</i>	50	0	2	Bahnameir(Jahani)	34
<i>Aphelenchoidessp</i>	0	0	1	Bahnameir(Jahani)	35
<i>Aphelenchoidessp</i>	0	0	2	BahnameirAdelian)	36
<i>Aphelenchoidessp</i>	0	0	2	Bahnameir(Adelian)	37
<i>A. fragariae</i>	50	0	3	Bahnameir(Jahani)	38
<i>Aphelenchoidessp</i>	0	0	3	Bahnameir(Jahani)	39
<i>Aphelenchoidessp</i>	0	0	2	Kiakola(Talaei)	40
<i>Aphelenchoidessp</i>			83		total

IV. DISCUSSION

Aphelenchoidesfragariae (RitzemaBos, 1890) Christie, 1932

Female: vermiform after the stabilization slim body straight up toward the abdomen is a little curvature. Head flat and fairly long with rounded edges, the cuticle network weak, almost on par with the body. Stylet is narrow, small knobs and clear. Lateral field is like a two vertical lines which is more obvious in the middle of body. The beginning of esophagus is narrow, the middle bubble of esophagus is clear and it is a round-like which fill the cross of body. Esophagus glands are long and overlap with intestine. Nervous ring surrounded the beginning of Isthmus. Excretory and secretory pore are at the same nervous ring or near dorsal side of it. Reproduction system consisted of sexual tube which toward the front of the body is stretched. Oocytes are existed in one side. Vulva slit and slightly excrescent, Uterine dorsal sac are long and more than half the distance to the vulva is vagina. The Long tail cone which in terminal leads to single simple appendage.

Male: Stylet, head & esophagus are like the female. Slim body Tail curved bow with the angle of 45 to 90 has the terminal is a small appendage. Sexual tube towards the beginning of body which the spermatocytes are in the same row. Three pairs of semi-abdominal papilla tail exist after excretory genital pore. Rose thorn-like speculum which the head and crown are growth showed in figure 1. Morphology characteristic and *Aphelenchoides* Morphometry sample which collected from strawberry plant are matching with *A. fragariae* [11]. These species of aerial parts and the soil are collected from Bahnamir that are the first report in Iran. Profiltration of D2- D3 gene 285 causes the production of section with the length of 744. Nucleoid order of this pieces compared with the storage sequences in the bank of gene, and show 98% similarity with the *A. fragariae* which storage in the bank of gene and with number of Eu3256840 [12].

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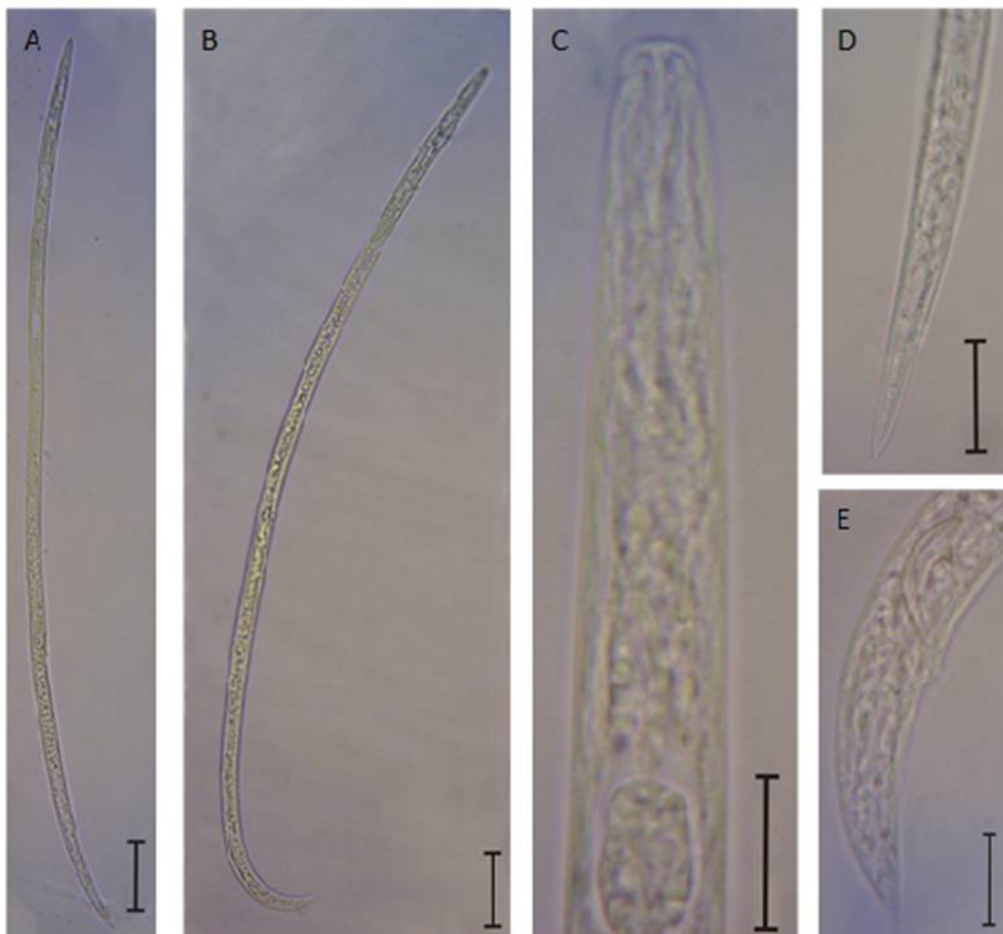
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This sample separated from Bahnamirregieon.

Figure 1. Separated *Aphelenchoides fragariae* from Strawberry;



A: Female Nematodes, B: male Nematode, C: Beginning of female Nematodes body, D: tail of female Nematode, E: tail of male Nematode. DXE= 10, AXB = 100 μ m.

Aphelenchoides snchaleos (Hoopera & Ibrahim, 1994).

Female: Vermiform, after the stabilization is curved a little to the abdomen. It has relatively short head with rounded edges, partly down with compared of body and has the weak cuticle network. The style of it is narrow, the cone is somewhat shorter than the cylinder, and it has clear and weak knot. The middle side of esophagus has the wounded body in which the central valve, long esophagus gland and has overlapped with gut. There are four longitudinal lines on the lateral sides which separated from each other and it is very clear. Reproduction system has one sexual tube which forward front of body. The vagina has a transverse slot and this tube has a transverse slot and the back bag of womb is very long. The rounded tail bind to central of body (figure 2). Male: the head and esophagus has the total characteristic of female which has the small body, the tail which bend to the abdomen and is rod-like shape with the angle of 45 to 90 and at the end it has needle-shaped. Sexual tube has a testis to forward of body. The head and miter of Speculum growth completely. (figure 2). Three pairs of Semi-abdominal sexual papillae saw after the excretory genital pore (figure 2).

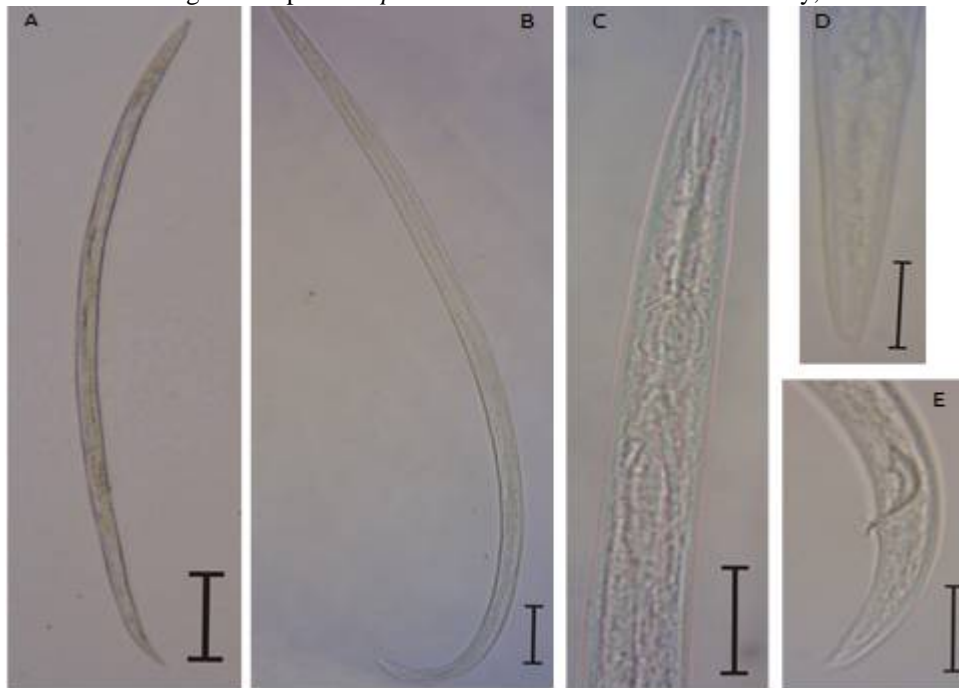
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Figure 2. Separated *Aphelenchoides nechaleos* from Strawberry;



A: Female Nematodes, B: male Nematode, C: Beginning of female Nematodes body, D: tail of female Nematode, E: tail of male Nematode. DXE= 10, AXB = 100 μ m.

Morphology and morphomery characteristic of *A. nechaleos* species which separated from strawberry plant compared with the characteristic of original species, most of species index of Iran are consisted with them. But it has a little difference with them which showed that the additional line existed in the middle of body and it can differentiate with optical microscope, but study with the scanning microscope showed that. This line; but explanation showed that there are 4 types of lateral line. Another differences in the length of spicule which the species of Iran has the spicule with long lateral and ventral side (lateral side 24-26 μ m vs. 15-20 μ m; ventral side 12-15 μ m vs. 9-12 μ m), species of *A. nechaleos* collected from the Strawberry aerial parts (20 Nematodes; Soil(100 Nematodes) which existed in Bahnamirand identified in Mazandaran province. This species is reported for the first time from Iran. But strawberry plants as a new host species is introduced to the world.

Aphelenchoides macromucronis (Slankis, 1967).

Female: vermiform and relatively thick, after stabilizing the head and the tail is curved slightly toward the abdomen. Head with mean growth depressed compare to the body. The con region is shorter than cylinder and the endless has good growth. Longitudinal line and lateral field consisted four line and it is very clearly. There is one sexual tube that stretch forward and continue to the end of esophagus, beginning of it has retroflexion sperm storage bag identified and it is spherical. Genital pore has transverse slot. Lateral bag of womb is long and it is equal or longer than the half of distance between genital and anus opening. The tail has conical shape and it is connected to the thick tubercle which

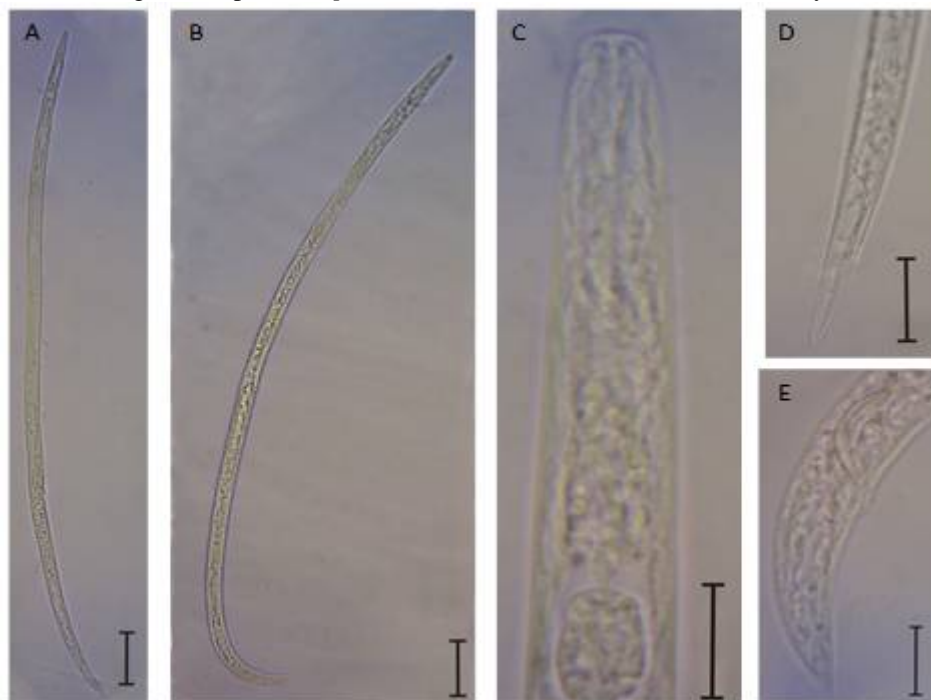
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Figure3. Separated *Aphelenchoides macromucrons* from Strawberry;



A: Female Nematodes, B: male Nematode, C: Beginning of female Nematodes body, D: tail of female Nematode, E: tail of male Nematode. DXE= 10, AXB = 100 μ m.

Male: A total characteristic is like female, reproductive organ extends towards the front of the body. Spicule growth completely. Spicules are fully developed, the bulging wand apex, the crown (rostrum) with good growth towards the abdomen is slightly raised. The ventral angle is longer than dorsal. The anus opening is outstanding (figure 3). The species in this research collected from strawberry with 500 nematodes that separated from aerial parts of plant in the Bahnamir, Kiakola with 450 nematodes in the soil and 20 nematode in the aerial parts, and Aznova with 10800 nematodes in the soil and 2000 nematodes from aerial parts are separated and identified.

V. CONCLUSION

We identified *Aphelenchoides* species in this examination consisted of *A. fragariae*, *A. nechaleos*, *A. macromucrons*. All three species of identified *Aphelenchoides* is first time that reported from strawberry plant of Iran. Among the identified species, *A. macromucrons* were separated from large number of region.

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