In the name of God



Islamic Republic of Iran

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Study on some plant parasitic nematodes of sugarcane in Khuzestan province of Iran

H. Moazzen Rezamahalleh[1], E. Shokoohi[2], N. Nasirpoor[1] and A. Mehrabi Nasab[2]

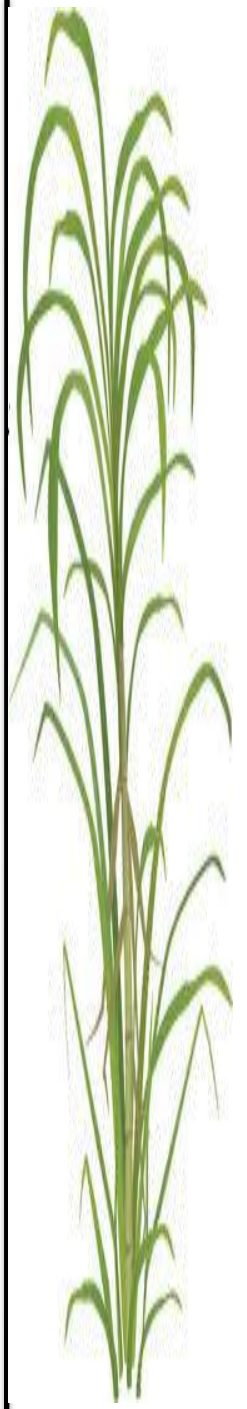
[1]Sugarcane and by Products Development Research and Training Institute, Khuzestan, Iran

[2]Plant Protection Department, College of Agriculture, Shahid Bahonar University of Kerman, Iran



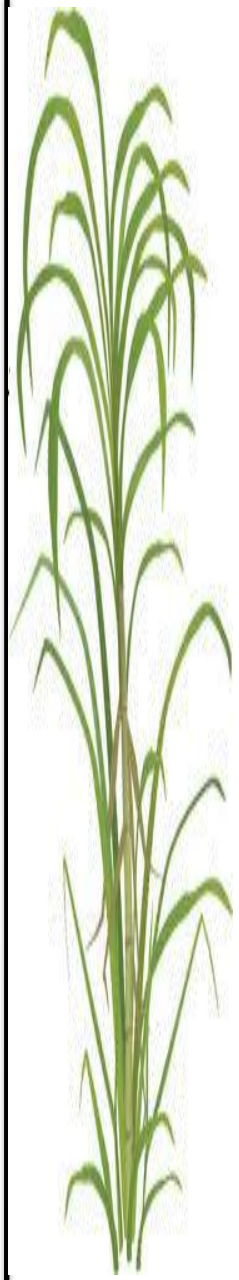
7-11th May 2012

NANNING - CHINA



- **Sugarcane is a tropical plant from poaceae family and saccharum genus.**
- **Genus saccharum, has speices with different characteristic that the distribution of S.officinarum caused sugarcane development in tropical and semi-tropical region.**

Sugarcane and its by-product are applied in food and pharmacy industries, chemical industries, distilling and fermenting industries, feedstuff industries, paper and wood industries.

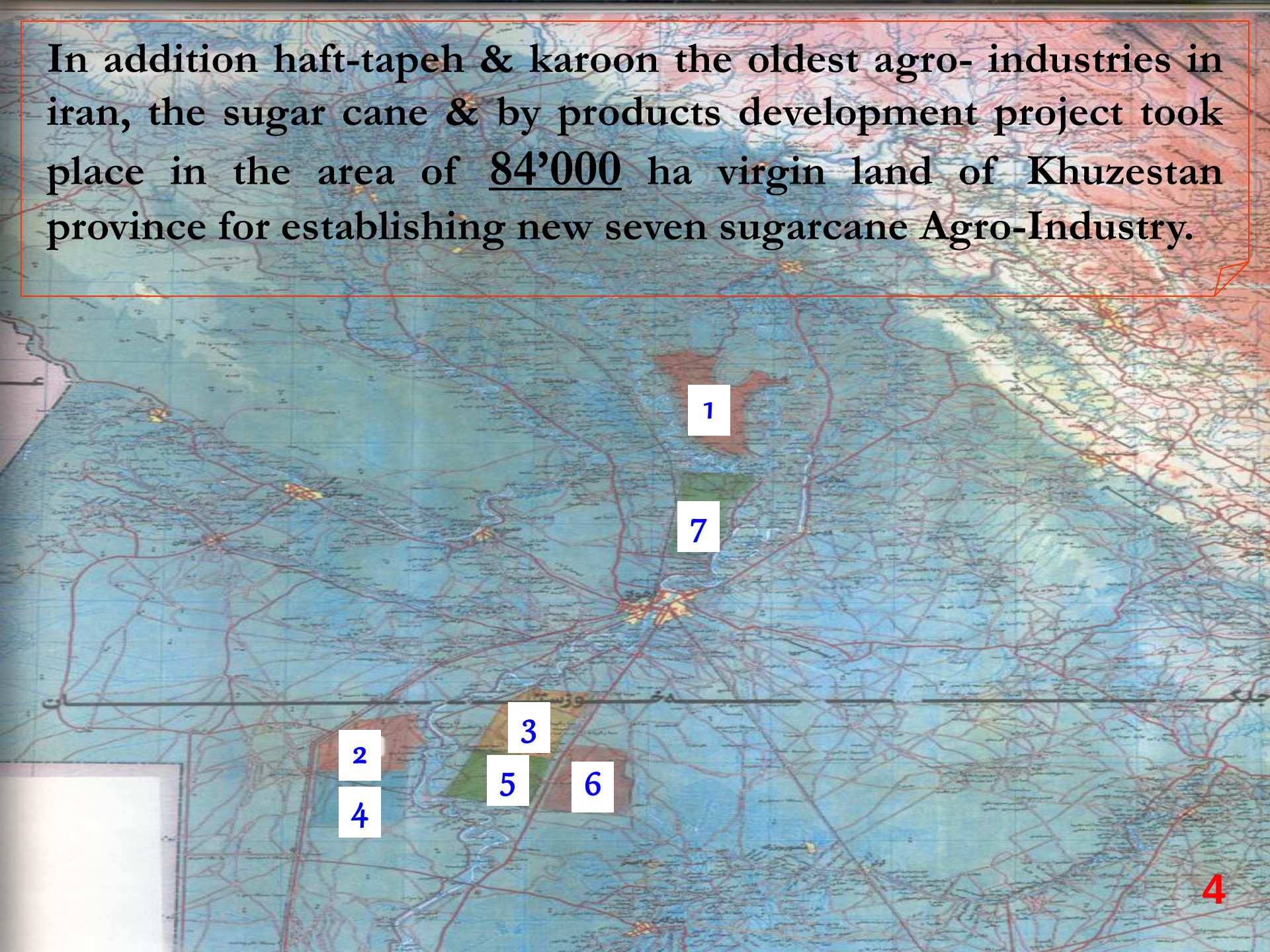




Islamic Republic of Iran lying on 25° – 38° Northern latitude and 44° – 60° Eastern longitude. The country has different climate conditions from cold-dry to Hot-humid with 1'648'000 km² area.


Geographical location
Khuzestan province is located in the south- west of Iran and sugarcane plantations lie on latitude of 31 to 32 N and a longitude of 48 E. (at elevation of 6 to 80 m above sea level).
Khuzestan
ARMENIA
AZERBAIJAN
Baku
CASPIAN SEA
TURKMENISTAN
Ashkhabad
TURKEY
Khvoy
Tabrīz
Ardabīl
Sabalān 15,783 ft.
Lake Urmia
Orūmīyeh
Rasht
GOLESTĀN NATIONAL PARK
Atrek
Irbīl
Mahābād
Zanjān
Āmol
Sanandaj
Kirkūk
Tehran
Hamadān
Qom
Bakhtarān
Arāk
Kāshān
Baghdad
Khorramābād
IRAQ
Euphrates
Behbehān
KUWAIT
Kāzerūn
Kuwait
Būshehr
Persian Gulf
SAUDI ARABIA
BAHRAIN
Al-Manāmah
QATAR
Doha
Abu Dhabi
U.A.E.
OMAN
Strait of Hormuz
Gulf of Oman
Jaz Murian
0 150 300 mi
0 150 300 km
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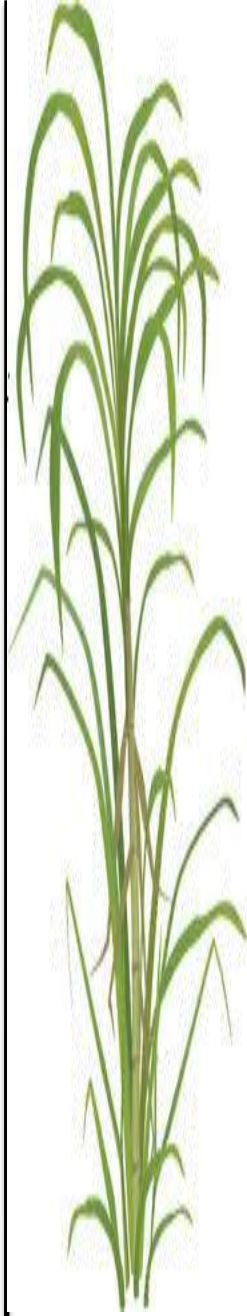
In addition haft-tapeh & karoon the oldest agro- industries in iran, the sugar cane & by products development project took place in the area of 84’000 ha virgin land of Khuzestan province for establishing new seven sugarcane Agro-Industry.



According to increasing sugarcane planted area and its economic importance, distinguish and control of its limited factors is necessary.

Sugarcane diseases is one of the most important factors in decreasing the yeild.

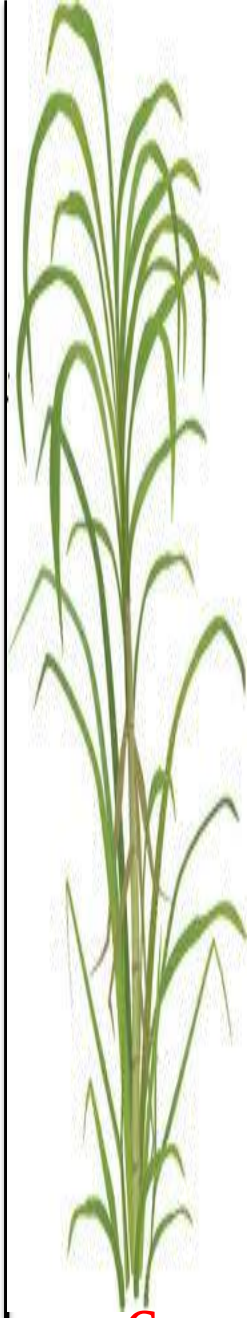




Sugarcane diseases

1-Sugarcane fungi diseases
2-Sugarcane bacterial diseases
3-Sugarcane viral diseases
4-Sugarcane phytoplasma diseases
5-Flowered parasitic plants
6-Disease because of sugarcane nematodes





Nematodes

Nema =Thread todes = Likeness

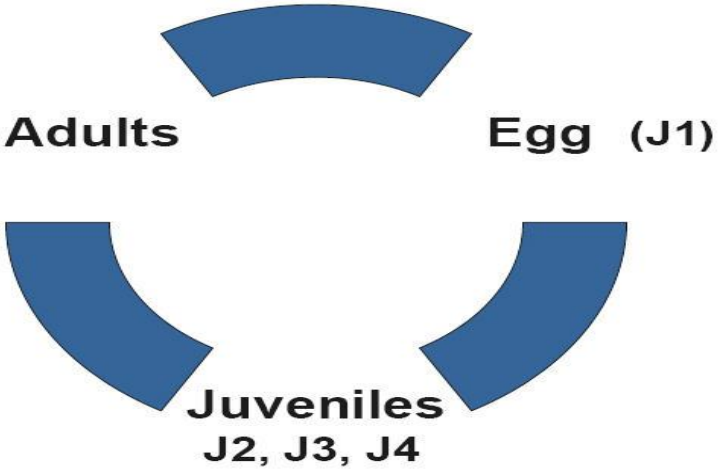
Nematod is named to a group of vermiform organisms(some genus females spherical and swollen) with smooth and non-segmented body.

Mostly are narrow, elongated, cylindrical and their body is covered with cuticle.

The nematodes have digestive, reproductive, nerve, secretory-excretory system and have no respiration and blood system.



The life-cycle of nematod is egg, four larva stage and adult.



Each nematod has four molting stage and the first molting usually conducts in the egg. So second stage larva emerge from the egg.



Nematodes Important

Damage value depend on genus, genus population, host susceptibility and environmental factors and it varies from 1 percent to lost whole the yeild.

According to global evaluation, the average of damage value of plant parasitic nematodes to forty main crops in the world, that are important in the case of food and economically was 12.3 precent of total yeild that its economic value is evaluated one hundred miliard dollars(sasser,1989).



Sugarcane Nematod

- **310 speices from 48 nematods genus are reported from sugarcane fields of the world.** (Cadet & Spaull,2005)

- **The speices of Pratylenchus and Meloidogyne are majority nematodes of sugarcane field in most sugarcane area of the world** (Cadet & Spaull,2005).



Materials and Methods





The diseased plant are chlorotic and stunted, and yellow stripes show on the young leaves while older leaves appear healthy.







Processing of samples

According to sugarcane planted area and field distribution in khuzestan province first step of sampling was conducted in sixty field from march of 2009 till aprill of 2010.

Ten points were selected at each field through zigzag pattern and then from the rhizosphere(10-30cm) of weak and yellowish bushes sampling were conducted.

Samples were always drawn randomly and a complex sample of 250 ml was drawn as representative of the field

In addition of soil sample, the sugarcane roots were gathered at each point.



During March-May 2011 second step of sampling was conducted to determine primary population of nematodes in 60 fields from south and north agro – industries of khuzestan.









Extract nematodes from samples

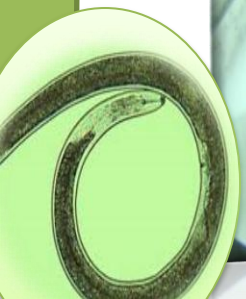
The nematodes of samples were extracted through two steps by the centrifugal-flotation method (De Grisse, 1969).











Killing and Fixing

The nematodes after killing and fixing with formaline in three steps and gradually transferred to glycerine.

Fixativ 1

1-Formalin(40% formaldehyd)	10 ml
2-Glacial acetic acid	2 ml
3-Glycerine	2 ml
4-Distilled water	88 ml

Fixativ 2

1-Glycerine	5 ml
2- Etanol %96	95 ml

Fixativ 3

1-Glycerine	50 ml
2- Etanol %96	50 ml

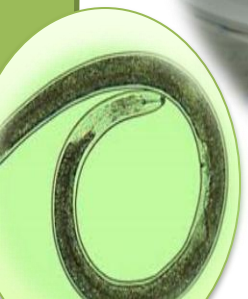












Permanent slide preparation





Permanent slides of nematodes speciments were prepared by transfering nematode speciments from the fixativ to dehydrated glycerine



Measurment and draw the picture

In order to identification nematodes, differents parts of the body was measured and their picture was drawn by means of binocular microscope equipped with drawing tube.



The measured samples were selected from one population, and their numbers was depended on the abundance of gathered nematodes varied from 4- 20 nematodes.



Results



Order: Tylenchida (Orley, 1880) Thorne, 1949)

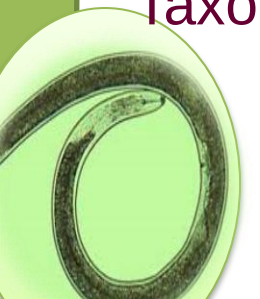
In this study, it is followed from Maggenti et al.,1987 classification which is approved by most of the nematologists.

In this survey, 4 species, *Meloidogyne javanica*, *Pratylenchus zea*, *Tylenchorhynchus annulatus* *Helicotylenchus exallus* were gathered and identified.



	Sub order	Over family	Family	Genus & Species
Tylenchida	Tylenchina	Tylenchoidea	Hetroderidae	Meloidogyne javanica
Tylenchida	Tylenchina	Tylenchoidea	Pratylenchidae	Pratylenchus zeae
Tylenchida	Tylenchina	Tylenchoidea	Hoplolaimidae	Helicotylenchus exallus
Tylenchida	Tylenchina	Tylenchoidea	Belonolaimidae	Tylenchorhynchus annulatus

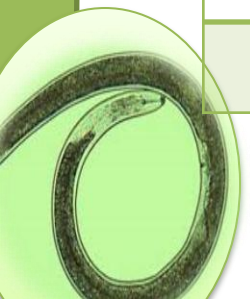
Taxonomical situation of collected speices from khuzestan sugarcane fields in maggenti et al. cassification



N	Species	distribution percent
1	*Meloidogyne javanica* (Treub, 1885) Chitwood, 1949	90
2	*Pratylenchus zeae* Graham, 1951	50
3	*Helicotylenchus exallus* (sher, 1966.)	80
4	*Tylenchorhynchus annulatus* (Cassidy,1930) Golden, 1971	60

List and distribution percent of indentified nematodes in khuzestan sugarcane fields

Helicotylenchus spp.		***Pratylenchus spp.***		***Meloidogyne spp.***	
RF=Pf/Pi	1.8	RF=Pf/Pi	0.9	RF=Pf/Pi	1.3



Meloidogyne (Goeldi, 1892)

Head and stylet weak
Hyalin developed
Oesophagous overlap intestin ventrally
Root endoparasitic
Polyphage and sedentary



Meloidogyne javanica (Treub, 1885) Chitwood, 1949

Mature female body pear-shaped with particular neck, milky colored, end of the body(vulval and anus region) is the same level as other part of the body or alittle outstanding.cephalic framework with moderate grow, stylet raise quitely well, the conus part is averagely 50% of total longht.

Lateral fields with two clear paralel lines. Phasmids are located between lateral fields at distance as vulval width or less.

Male: not observed

Larva body is narrow. Cuticle with transverse, striae, lateral fields have four longitudinal striae, narrow stylet with lateral and round knobes. Oesophagous median buld is large and has crescent valve at center.

The characteristics of studied population were similar to Meloidogyne javanica with the original description given by (Jepson,1987) and (Eisenback & Triantaphyllou, 1991).



Meloidogyne javanica (Treub, 1885) Chitwood, 1949

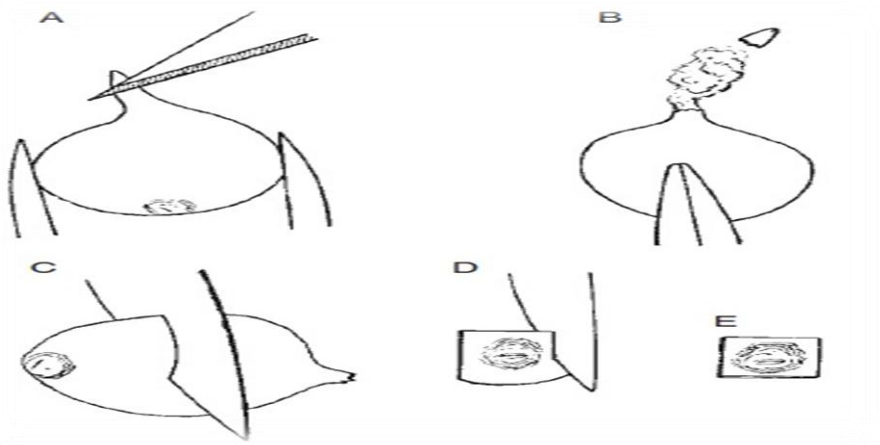
characters	**AVG ± STD (MIN-MAX)**
L	386 ± 14.4 (366-410)
a	30.2 ± 0.6 (29.1-31.0)
b	5.3 ± 0.1 (5.1-5.5)
b'	3.5 ± 0.1 (3.5-3.6)
c	8.0 ± 0.4 (7.6-8.7)
c'	5.5 ± 0.4 (5.0-5.8)
Stylet	12.8 ± 0.3 (12.4-13.2)
Conus	7.6 ± 0.6 (6.7-8.2)
m (conus/stylet %)	59.4 ± 3.7 (53.7-63.4)
DGO	2.3 ± 0.1 (2.1-2.4)
o	17.8 ± 1.1 (16.3-19.0)
Head-Oesophagus end	109 ± 3.4 (103-113)
Oesophagus	73.6 ± 2.1 (71.2-77.1)
Head-Median bulb	54.7 ± 2.8 (51.5-58.9)
MB	74.4 ± 3.9 (71.7-82.7)
Median bulb height	12.9 ± 0.7 (11.9-13.6)
Median bulb width	6.9 ± 0.5 (6.2-7.8)
S. E. pore	71.5 ± 4.5 (66.2-77.2)
Hemizonid	68.3 ± 4.2 (63.2-74.3)
Nerve ring	74.1 ± 2.6 (71.2-78.4)
Tail	48.2 ± 0.9 (47.3-49.2)
Body width	12.8 ± 0.4 (12.1-13.3)
Vulval body width	13.1 ± 0.3 (12.7-13.5)
Anal body width	8.8 ± 0.6 (8.1-9.6)
Head width	4.9 ± 0.4 (4.3-5.4)
Head height	2.6 ± 0.2 (2.3-2.8)
Annule width	1.0 ± 0.1 (0.9-1.2)
Head-anus	338 ± 14.7 (319-363)










A
B
C
D
E



Pratylenchus Filipjev, 1936

It differentiated from other genus of family because of an anterior ovary, resolution of posterior branch of gonad to post vulval sac, oesophagous overlap intestine ventrally, no deirid and no secondary biformis



Pratylenchus zeae (Graham, 1951)

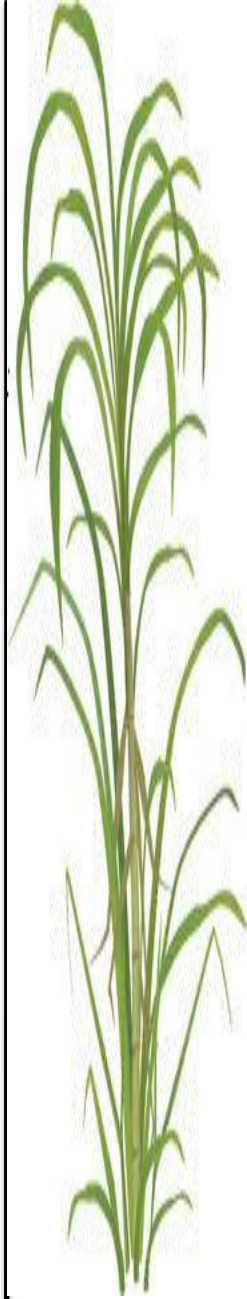
Female charactristic: body slender, marked by very faint annules, cephalic region with three striae along the body, (some have four striae at a side and three striae at another side).

Lateral fields is elongated to beyond the phasmid with four incisures. Lateral lines band show a slight irregularity in the mid-body region.

Hemizonid is located two annulae befor secretory-excretory pore. oocytes is double row- oviduct indistinct, Uterus short, Spermatheca round and small, Phasmid is sited next to mid tail. Tail becomes narrow toward the tail tip.

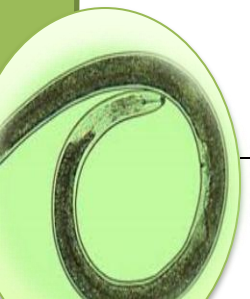
The characteristics of studied population were similar to Pratylenchus zeae with the original description given by (sher,1970) and (loof, 1991).

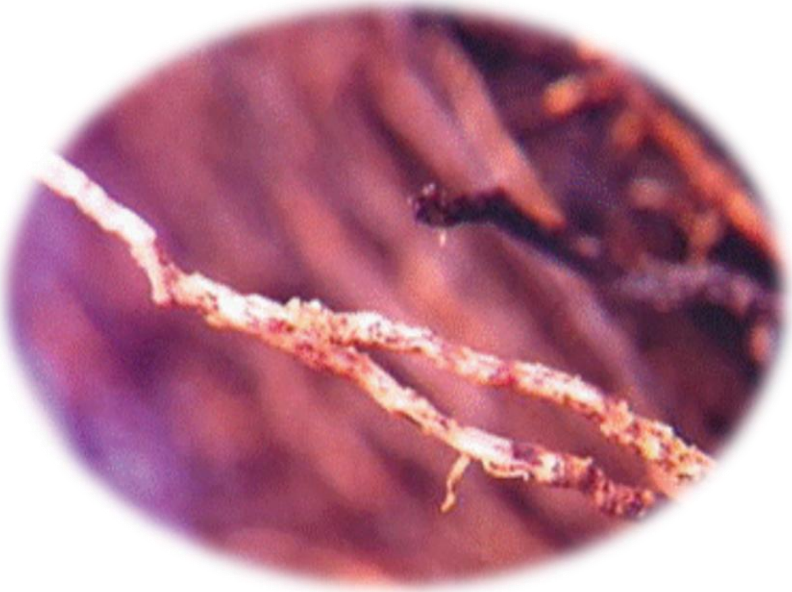




Pratylenchus zeae (Graham, 1951)

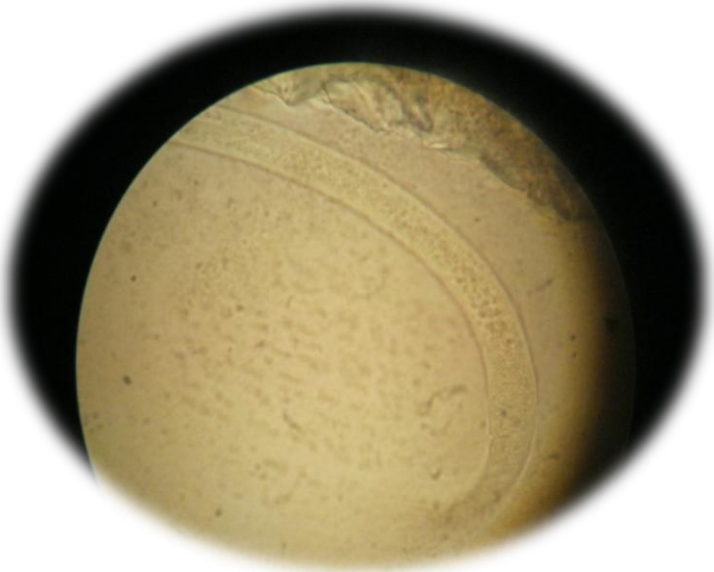
Characters	Ahvaz
	AVG±STD(MIN-MAX)
L	407±52.2(347.4-505.2)
a	28.1±5.6(23.1-41.3)
b	5.7±1.3(4.8-8.9)
c	18.8±2.1(16.7-24.1)
c'	2.3±0.2(1.9-2.6)
V	71.2±3.4(66.5-77.0)
Stylet	14.6±0.8(13.2-15.8)
Conus	7.1±1.2(4.0-8.5)
m (conus/stylet %)	48.9±8.5(26.4-55.7)
DGO	3.6±0.5(3.1-4.3)
o	25.0±3.9(19.5-29.9)
Head-Oesophagus end	106±12.5(83.2-122.0)
Oesophagus	72.6±9.0(56.6-87.2)
Head-Median bulb	45.1±2.7(41.4-50.6)
MB	62.7±5.7(56.9-73.1)
Median bulb width	8.5±0.7(7.5-9.5)
S. E. pore	68.2±5.0(62.8-77.0)
Hemizonid	70.7±12.0(61.8-100.4)
Nerve ring	59.1±6.3(50.4-74.1)
Head-vulva	290±40.5(233.7-354.7)
Tail	21.7±2.0(17.6-24.4)
Body width	14.8±2.3(- .8-18.9)
Vulval body width	15.8±1.3(14.7-17.5)
Anal body width	9.5±0.8(8.6-10.8)
Vagina	5.7±1.4(3.4-7.9)
Head width	7.0±0.4(6.4-7.5)
Head height	2.4±0.4(2.0-3.4)
Annule width	1.1±0.1(0.9-1.2)
Head-anus	373±42.0(315.8-438.9)
Tail Anulli	21.8±2.5(18.0-26.0)
Stylet Knobes with	3.9±0.3(3.3-4.4)













Helicotylenchus (Steiner, 1945)

This genus is differentiated of other genus of family according to some characteristic such as: oesophagous over-lop intestine ventrally-dorsally, low distance between stylet nodes and oesophagous dorsal gland valve, small plasmid on tail or out of it.

The spiral nematode, Helicotylenchus was the second ubiquitous nematode on sugercane in surveyed areas.



Helicotylenchus exallus (sher, 1966.)

Female: Head hemispherical with 4-5 labial annules along the body. Stylet knobes distinct and round. Valve of median bulb distinct, Nerve ring at first half of isthmus, Hemizonid about 1-3 striae and on the excretory-secretory pore,Oesophagus and intestine overlop laterally-ventrally. Oocyte arranged in single row. Lateral fields with four longitudinal striae.

Male: Stylet small(14) with weak knobs. Spicule and gubernaculum simple and without any peg.Bursa elongate terminally.

Specific characteristics of H. exallus are excretory- secretory pore is in front of cardia, phasmid is located anteriorly to cardia and anus, tail variation.

The characteristics of studied population were similar to Helicotylenchus exallus with the original description given by (sher,1966) and (Krall, 1990). And (Fotedar & Kaul 1985).



Helicotylenchus exallus

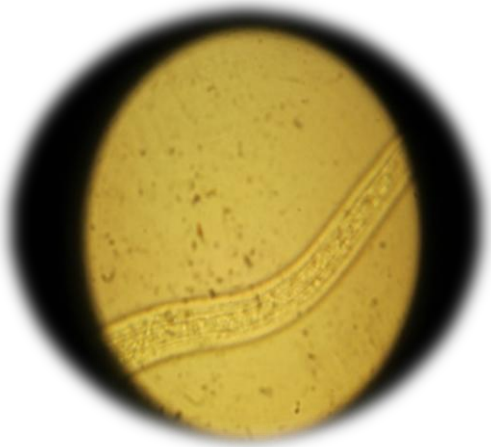
Characters	**Ahvaz**
L	685.7 ± 44.6 (650-735.7)
a	26.5 ± 6.2 (21-33.3)
b	5.2 **±0** .1 (5-5.4)
G1	16.1 ± 2.8 (13.1-18.7)
G2	18.4 ± 5 (15.1-24.2)
c	24.77± 7.5 (19.2-33.3)
c'	1.6± 0.2 (1.5-1.9)
V	60.3 ± 3.5 (56.3-62.7)
Stylet	24.5 ± 1.6 (23.5-26.4)
m	48.1 ± 3.2 (44.4-50)
MB	56.1 ± 2.3 (53.4-57.4)
Procorpus	66.6± 6.9 (58.8-72)
Oesophagus	130.8 ± 3.8 (127.9-135.2)
O	14.3 ± 1.8 (12.5-16.2)
Excretory pore	98.5 ± 16.3 (83.8-116.1)
Nerve ring	97.5 ± 12.5 (88.2. -111.7)
Tail length	28.9 ± 6.1 (22-33.8)
Anal body width	17.1 ± 2.2 (14.7-19.1)
Maximum body width	26.4 ± 4.4 (22-30.8)

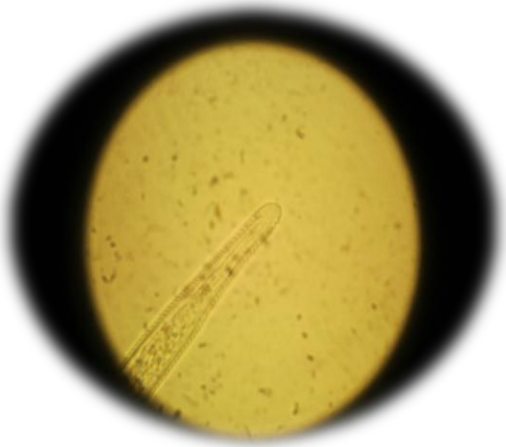




Tylenchorhynchus (Cobb, 1913)

This genus is differentiated from other family genus because of conoid to cylindrical tail and 2-5(usually 4) longitudinal striae at lateral fields.





The stunt nematode, Tylenchorhynchus. a moderate(80-400/100 ml soil) population of this nematode was found to be correlated with stunting of canes and yellowing of foliage .



Tylenchorhynchus annulatus(Cassidy,1930) Golden, 1971

Lip region off-set and 2-3 annules, Stylet 17-21 µm long with laterally directed knobs. Tail termines smooth, sub-cylindrical with a hemispherical terminus bearing.

It species can be differentiated because of its continuous head, larger number of tail annules, presence of spermatheca, and males. it can be distinguished from T. mashhoodi because of longer spear, lower ‘b’ value and a larger number of tail annules.

The characteristics of studied population were similar to *Tylenchorhynchus annulatus* with the original description given by Zafar-Handoo,2000.



Tylenchorhynchus annulatus

Characters	**Ahvaz**
L	793.8 ± 98.7 (697-894.7)
a	29.4± 0.4(28.9-29.8)
b	5.3 **±0** .3 (5-5.7)
G1	21.8 ± 3(19.7-25.3)
G2	23.9 ± 0.5(23.4-24.4)
c	13.7± 0.8(12.8-14.5)
c'	3.4± 0.3 (3-3.7)
V	51.5 ± 3.1 (47.8-53.3)
Stylet	19.1 ± 1.4 (17.6-20.5)
m	50.8 ± 8.1 (41.6 -57.1)
MB	45.8 ± 1.8 (43.8-47)
Procorpus	60.2± 2.9 (57.3-63.2)
Oesophagus	147.5 ± 8.3 (138.2-154.4)
O	23.1 ± 1.7 (21.4-25)
Excretory pore	111.7 ± 2.9 (108.8-114.7)
Nerve ring	97.5 ± 6.6 (91.1. -104.4)
Tail length	57.8 ± 5.9 (54.4-64.7)
Anal body width	16.6 ± 1.6 (14.7-17.6)
Maximum body width	26.9 ± 3.7 (23.5-30.8)



According to results, most distribution is related to *Meloidogyne javanica* and *Helicotylenchus exallus*. As these nematodes are very important and on the other hand, sugarcane is one of main economic crops of khuzestan province, so the pathogenisity test is being done.

Applied morphological methods to identification and isolation of some speices is not enough, and molecular methods should be done to have reliable results.





Thank You