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THE INCIDENCE AND PREVALENCE OF ROOT-KNOT NEMATODE SPECIES (*MELOIDOGYNE* SPP.) ASSOCIATED WITH DIFFERENT DICOTYLEDONS ORIGINATED FROM TWO VEGETABLE CROPPED AREAS, VĂRĂŞTI (GIURGIU), AND BĂLENI (DÂMBOVIȚA)

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Abstract

Although the vegetable fields is reduced in Romania, the production losses remain high and this concern is not owed only to fungui, viruses, bacteria or insects, but also to nematodes, these last organisms being less known and acknowledged. Meloidogyne incognita (Kofoid & White, 1919) and Meloidogyne hapla (Chitwood, 1949) are considered important parasitic nematodes, but quite little studied in Romania, for different species of dicotyledon vegetables and this article demonstrates and compares the development and reproduction of these two species related to one of the most important vegetable cultures from the economic point of view. It seems that the Meloidogyne hapla species especially prefer the species from the Apiaceae family, unlike those belonging to Meloidogyne incognita which develop a more intense shock on Brassicaceae and Solonaceae botanic family species. However, the eggs masses detected for both root-knot nematodes species show a unitary type of distribution at the surface of the of roots cortical area of all analyzed vegetable species. A contamination with the Meloidogyne incognita species, unusually high, has been noticed for the first time in our country in Brassica oleracea species.The diagnosis through biomorphometry on semi-permanent microscopic preparations, completed with the molecular biology techniques (restriction fragment length polymorphism - RFLP$_s$) led to the conclusion that the twocategories of diagnosis methods can be considered as being complementary.

Key words*: diagnosis, galles, larves, root-knot nematodes (Meloidogyne* spp.*).*

INTRODUCTION

The genus *Meloidogyne* Göldi 1892 or the root-knot nematodes (RKN) consists of sedentary, polyphagous root endoparasites (Sharon *et al*. 2007). More than 100 species have been reported worldwide (Karssen & Moens 2013). Although nematodes from the *Meloidogyne* family were reported for the first time in Romania, at the end of the 40s, under the name of "root worms" or *Heterodera maroni* Cornu (Manolache *et al*. 1949), the studies elaborated along the time, focused on the control issues, attack methods and to the produced damages, and less on the attacked host plants. At the same time, there are little references regarding the share of *Meloidogyne* species, in the large fields cultivated with vegetables in our country. The nematode is very harmful causing essential losses especially in the tropical and subtropical areas. Unfortunately, our country is not avoided by their more or less aggressive attack, despite its location in the temperate area.

The root-knot nematodes (RKN) are making the object of large studies regarding the aggression level of the attacks and the management of such attacks, having as final purpose, to reduce the application of chemical or non-chemical (in tests) nematocides. In order to reach this, a new approach is needed, based on a correct identification of *Meloidogyne* species.

Therefore, the objectives of this study were to establish the incidence and the prevalence of RKN species, in two important vegetable fields, the vegetables host associated with the attack of these nematodes, the density of the last ones, as well as the combination of the classic diagnosis methods with those of molecular biology.

MATERIALS AND METHODS

The nature of the samples was constituted of soil and roots resulted from two important vegetables fields, Vărăşti, respectively Băleni. Vărăşti commune is located in the passage of Săbarului valley, at the east limit of Giurgiu County (South-Eastern Romania), at 30 km from Bucharest. Băleni commune is located in the south side of Dâmbovița County (Central-Southern Romania) situated at a distance of around 20 km from Târgovişte Municipality. Both areas are under the incidence of the temperate-continental climate, characterized by very hot summers, moderate rainfalls and not so cold winters, with rare winter blizzards and frequent warming periods.

The biotic and abiotic diversity allows the cultivation of a large variety of vegetables, on protected fields and especially on agrarian fields, providing an important amount from the vegetables production of the country.

However, none of both fields was avoided by the pest attack, including the root-knot nematodes (RKN).

The samples (mixture of soil and vegetables roots) were collected within April 2014 – November 2014 and the identification of root-knot nematodes species belonging to *Meloidogyne* family was made on stages.

There were mainly chosen plants with low fructification, dwarfing phenomena and different levels of wilting.

The soil was collected from a depth of 15-20 cm, using a hand shovel, following a zig-zag model. The adherent soil on roots collected from 11 micro-farms (6 from Băleni - 5ha, respectively 5 micro-farms from Vărăşti – 6ha) was carefully shacked, attentively observing the roots (presence/absence of galls). Then two samples of 1 kg soil and roots/ha were collected. The samples were divided in sub-samples of around 200 g. This sub-division was necessary for a better laboratory processing.

The samples were stored in polyethylene bags at temperatures of 7 - 10˚C, until their processing.

The storage of the last ones was performed on stages for a period of 5-7 days.

The nematodes extraction from soil was performed using the Cobb's method, through sieving and decantation and afterwards using the Baermann modified method (Southey, 1985). The nematodes were collected in aqueous suspension during no more than three days, numbered on counting dish, using a binocular stereomicroscope (Leica MZ95) and the density was established as number of nematodes on 200 g soil.

To establish the density in roots, they were shacked to remove the adherent soil, carefully washed and cut in pieces of 1-2 cm.

The nematodes extraction was performed placing the roots (aprox 10 g) in hatching chamber to produce juveniles hatching from the eggs (McKenry and Roberts, 1985). After five days, the nematodes were numbered under a binocular stereomicroscope.

The perineal pattern was placed in glycerine, after the females were dissected from the roots galls to be identified (Jepson, 1987).The nematodes were killed in water at 70˚C, fixed in TAF and placed in glycerine, on a glass slide sealed with paraffin ring, for identification, using a stereomicroscope Leica DMLB with camera Leica DC300 and Leica DFC295 image-processing software. The severity of the attack on roots was evaluated on a scale from 0 to 5, as it follows: 1= 1-2 galls; 2= 3-10 galls; 3= 11-30 galls; 4= 31-100 galls; 5= over 100 galls (Taylor and Sasser, 1978).

The reception of the samples, their storage, the extraction of the nematodes, their preliminary examination, the microscopic preparations and the bio-morphometric identification were

performed at the Regional Laboratory of Nematology-Braşov while the bio-morphometric observations of species confirmation and diagnosis through molecular biology were performed at the Central Phytosanitary Laboratory–Bucureşti.

The RKN incidence for each culture (host plant), for each vegetable field, as well as the global (total) incidence, were calculated using the formula below (Hussain, 2012):

$$\text{Incidence (\%)} = \frac{\text{Total number of infected plants}}{\text{Total number of observed plants}} \text{ X } 100$$

The prevalence for each vegetable field was calculated using the formula below (Hussain, 2012):

$$\text{Prevalence (\%)} = \frac{\text{Total number of fields with root-knot nemaodes}}{\text{Total number of fields surveyed}} \text{ X } 100$$

The incidence of each RKN species (occurrence) for each vegetable field was calculated using the formula (Norton, 1978):

$$\text{Occurrence of specie (\%)} = \frac{\text{Number of sample within species}}{\text{Total number of samples observed}} \text{ X } 100$$

In terms of molecular biology analysis there were used juveniles of the two species, following the steps below. Therefore, DNA extraction was carried out using ten juveniles collected directly from samples.

The juveniles were crushed between a glass slide and the cover slip by gentle pressure.

The extract was recovered with 20 µL of lysis buffer (10 mM Tris pH = 8.8, 1 mM EDTA, 1% Triton X-100, 100 mg/mL proteinase K) incubated at 60°C for 1 h, then at 95°C for 10 min (Ibrahim *et al*., 1994). The ITS regions of rDNA were amplified using the forward primer 18S 5'-TTG ATT ACG TCC CTG CCC TTT-3' and reverse primer 26S 5'-TTT CAC TCG CCG TTA CTA AGG-3' (Vrain *et al*., 1992).The PCR mixture (total volume 25 µL) contained 1x buffer enzyme, 1, 5 mM $MgCl_2$, 0.6 µM of each primer, 1 U Taq DNA polymerase (MP Biomedicals), 0.2mM dNTPs (MP Biomedicals) and 5 µL DNA extract AMasterCycler Pro S (Eppendorf) was used for amplification, and the reaction consisted of a denaturation step at 94°C for 15 min followed by 45 cycles at 94°C for 15 s, 58°C for 30 s, 72°C for 1 min, and a final extension step of 10 min 72°C. Following PCR, 10 µL of the amplified product was analysed by electrophoresis in a 1% agarose gel.Amplified DNA was digested with DraI and RsaI restriction endonucleases (Fermentas and Promega) using an aliquot of 5 µL of the PCR product and 5 U of each enzyme, according to the manufacturer's instructions. Species-specific ITS–RFLP profiles for *Meloidogyne* were generated using these two restriction enzymes (Zijlstra *et al*., 1995).

Fragments were resolved by electrophoresis in 1,5-2% agarose gel. Data analysis was performed using GENi (Syngene) and 100 bp DNA Ladder (GeneRuler, Fermentas) as a molecular size marker.

Analyses of nematodes density were carried out using SPSS Statistics (Statistical Package for the Social Sciences, available online at https://statistics.laerd.com/spss-tutorials/one-way-anova-using-spss-statistics.php) and the Standard error calculation (available online at http://www.investopedia.com/terms/s/standard-error.asp).

RESULTS AND DISCUSSIONS

The most important vegetable cultures from Băleni (Dâmbovița County), namely Vărăşti (Giurgiu County) were studied: beet, celery, parsnip, carrot, parsley, lettuce, broccoli, cauliflower, cucumber, vegetable-marrow, tomato and pepper.

Among the 120 analysed samples, the majority composed of a mixture of soil and roots, 105 samples were positive for RKN.

From the total number of samples which were examined, from both regions, it resulted that the global incidence is 87.5 % infested samples with RKN, but there are differences concerning the incidence reported to different vegetables species.

Therefore, the incidence from Băleni area starts from 50% for *Lactuca sativa* and *Cucurbita pepo*, achieving the maximum percentage in case of *Apium graveolens* (Figure 1*), Pastinaca sativa* (Figure 2)*, Brassica oleracea* and *Brassica oleracea* var*. botrytis* (Table 1).

From Vărăşti area the incidence starts from 50% for *Beta vulgaris* achieving the maxim percentage to *Daucus carota* and *Petroselinum crispum,* among others (Table 2).

Figure 1. Infested roots – *Apium graveolens*

In Vărăşti area, the incidence for all positive (+) samples of vegetables is 88.33 %, unlike Băleni area, where this incidence was 86.66%. Although these differences between the two vegetable areas are not too significant, it is to be noticed the RKN incidence of 100% to the family members of *Brassicaceae* from Băleni area, along with a significant deformation of the roots.

Figure 2. Infested roots – *Pastinaca sativa*

This deformation is due the galls. Each gall usually contains three to six giant cells, which are due to substances contained in the "saliva" secreted by the nematode in the giant cells during feeding. The giant cell crush xylem elements already present but degenerate when nematodes cease to feed or die. In the early stages of gall development the cortical cell enlarge in size, later, they also divide rapidly.

Frequently other parasites can easily attack the weakened root tissues and the hypertrophied, undifferentiated cells of the galls (Agrios, 2005).

Table 1. The incidence of RKN (%) for crop host at Băleni – Dâmbovița
(+ present, - absent, RKN root – knot nematode)

Botanic family	Crop host	Total soil samples	+	-	Incidence RKN %
Amaranthaceae	*Beta vulgaris*	4	3	1	75
Apiaceae	*Apium graveolens*	4	4	-	100
	Pastinaca sativa	6	6	-	100
	Daucus carota	7	6	1	85.7
	Petroselinum crispum	7	6	1	85.7
Asteraceae	*Lactuca sativa*	2	1	1	50
Brassicaceae	*Brassica oleracea*	5	5	-	100
	Brassica oleracea var. *botrytis*	7	7	-	100
Cucurbitaceae	*Cucumis sativus*	3	2	1	66.66
	Cucurbita pepo	2	1	1	50
Solanaceae	*Lycopersicon esculentum*	11	10	1	90.90
	Capsicum annuum	2	1	1	50
Incidence of RKN/area 86.66 %					

Table 2**.** The incidence of RKN (%) for crop host at Vărăşti – Giurgiu
(+ present, - absent, RKN root – knot nematode)

Botanic family	Crop host	Total soil samples	+	-	Incidence RKN %
Amaranthaceae	*Beta vulgaris*	2	1	1	50
Apiaceae	*Apium graveolens*	3	3	-	100
	Pastinaca sativa	8	7	1	87.5
	Daucus carota	7	7	-	100
	Petroselinum crispum	5	5	-	100
Asteraceae	*Lactuca sativa*	1	1	-	100
Brassicaceae	*Brassica oleracea*	3	2	1	66.66
	Brassica oleracea var. *botrytis*	3	2	1	66.66
Cucurbitaceae	*Cucumis sativus*	4	3	1	75
	Cucurbita pepo	2	1	1	50
Solanaceae	*Lycopersicon esculentum*	13	13	-	100
	Capsicum annuum	9	8	1	88.88
Incidence of RKN/area 88.33 %					

The incidence of each species of nematodes (occurrence) in different cultures of host-plants, underlines the fact that *Meloidogyne incognita* species is predilected to *Solanaceae, Cucurbitaceae* and to *Brassicaceae* (Table 3). The *Meloidogyne hapla* species grow better on species of root vegetables, in other words species of vegetables of the *Apiaceae* family (Table 4). Occurrence of species *Meloidogyne hapla* at Băleni area is predominant (50%) unlike the species *Meloidogyne incognita* (36.66%). Occurrence of species *Meloidogyne incognita* is higher at Vărăș ti area (53.33%) while *Meloidogyne hapla* was found in less than 50% (31.66%). The index of the galls is a sign of the nematodes presence or absence in roots and implicitly in soil. This index indicates the severity of the attack. Therefore, this severity is maximum (5) to *Brassicaceae* family (Figure 3) from Băleni while the index of the galls is raised (4) to *Apiaceae* family (Figure 4) and *Solanaceae* families from Vărăşti.

The mixed RKN population are relatively common in the vegetables plantations, only that in our study they were found only to *Lycopersycom esculentum* specie from Băleni area.

Table 3. The incidence of each RKN species (occurrence) and the index of the galls at Băleni - Dâmbovița area (*M.i. = Meloidogyne incognita, M.h. = Meloidogyne hapla*)

Botanic family	Crop host	*M.i.*	*M.h.*	Non detected	Gall index
Amaranthaceae	*Beta vulgaris*	-	3	1	2
Apiaceae	*Apium graveolens*	-	4	-	4
	Pastinaca sativa	-	6	-	2
	Daucus carota	-	6	1	3
	Petroselinum crispum	-	6	1	4
Asteraceae	*Lactuca sativa*	-	1	1	1
Brassicaceae	*Brassica oleracea*	5	-	-	5
	Brassica oleracea var. *botrytis*	7	-	-	5
Cucurbitaceae	*Cucumis sativus*	2	-	1	3
	Cucurbita pepo	-	1	1	3
Solanaceae	*Lycopersicon esculentum*	7	3	1	4
	Capsicum annuum	1	-	1	2
	Occurrence RKN (%)	36.66	50	13.34	

Table 4. The incidence of each RKN species (occurrence) and the index of the galls at Vărăşti - Giurgiu area (*M.i. = Meloidogyne incognita, M.h. = Meloidogyne hapla*)

Botanic family	Crop host	*M.i.*	*M.h.*	Non detected	Gall index
Amaranthaceae	*Beta vulgaris*	1	-	1	1
Apiaceae	*Apium graveolens*	3	-	-	3
	Pastinaca sativa	-	7	1	4
	Daucus carota	-	7	-	4
	Petroselinum crispum	-	5	-	4
Asteraceae	*Lactuca sativa*	1	-	-	2
Brassicaceae	*Brassica oleracea*	2	-	1	1
	Brassica oleracea var. *botrytis*	2	-	1	1
Cucurbitaceae	*Cucumis sativus*	3	-	1	3
	Cucurbita pepo	1	-	1	3
Solanaceae	*Lycopersicon esculentum*	13	-	-	4
	Capsicum annuum	8	-	1	4
	Occurrence RKN (%)	53.33	31.66	15.01	

Figure 3. Infested roots – *Brassicaceae (Brassica oleracea*)

Figure 4. Infested roots – *Apiaceae (Daucus carota)*

The prevalence was of 100% in both vegetable fields, so that all studied fields were infested with root – knot nematodes.

At least 40 perineal patterns from each vegetable field were examined for better identification accuracy (Figure 5 and 6).

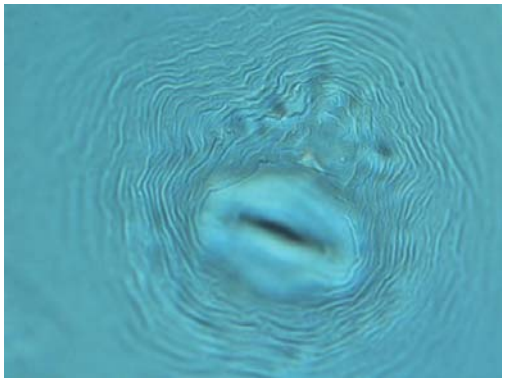

Figure 5. *Meloidogyne incognita* – female perineal pattern (100x magnification). Scale bar =20µm

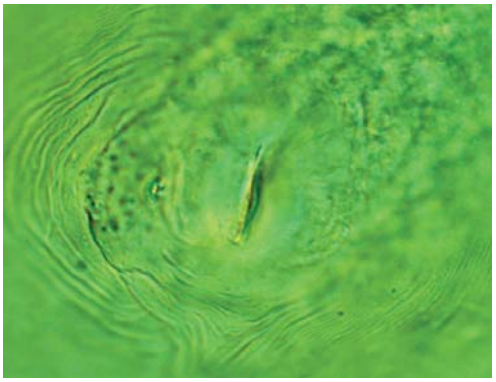

Figure 6. *Meloidogyne hapla* - female perineal pattern (100x magnification). Scale bar =20µm

Statistical analysis used fuctions Mean and ANOVA belonging program SPSS. Mean number of nematodes on roots was significantly higher at Băleni (11/10 g roots) compared to 8.25/10 g roots al Vărăș ti (Sig. 0.0006). At Băleni the total number of nematodes (soil+roots) was significantly higher compared to Vărăș ti (16.89)(Table 5).

At Băleni area the highest total mean number of nematodes was found at *Brassica oleracea* var. *botrytis* (134.8).

The highest total mean number of nematodes at Vărăș ti area was at *Cucurbita pepo* of which 42.2 nematodes/200 g soil and 51.4 nematodes/10 g roots (Table 6).

Table 5. Nematode population density per 200 g soil + 10 g roots of diseased crop host at Băleni (± Standard error)

Crop host	Positive samples (+)	Mean number of nematodes ±Standard error		
		200 g soil	10 g roots	Total
Beta vulgaris	3	6.53±0.40	6.73±0.34	13.27±0.61
Apium graveolens	4	9.40±0.76	10.75±0.48	20.15±0.97
Pastinaca sativa	6	3.37±0.34	3.27±0.35	6.63±0.49
Daucus carota	6	6.63±0.34	5.07±0.41	11.70±0.55
Petroselinum crispum	6	6.80±0.34	6.67±0.38	13.47±0.55
Lactuca sativa	1	2.40±0.51	10.60±1.81	13.00±1.38
Brassica oleracea	5	32.08±0.75	35.60±0.92	67.68±0.95
Brassica oleracea var. *botrytis*	7	61.60±1.03	73.20±1.46	134.80±1.07
Cucumis sativus	2	21.40±1.71	30.80±1	52.20±2.16
Cucurbita pepo	1	21±2.05	26.00±1.52	47.00±1.70
Lycopersicon esculentum	10	4.38±0.28	5.12±0.40	9.50±0.47
Capsicum annuum	1	2.43±0.27	2.83±0.31	5.26±0.41
Mean at Băleni	52	9.75±0.72	11.0±0.9	20.8±1.55

Table 6. Nematode population density per 200 g soil + 10 g roots of diseased crop host at Vărăș ti (± Standard error)

Crop host	Positive sample (+)	Mean number of nematodes ±Standard error		
		200 g soil	10 g roots	Total
Beta vulgaris	1	7.00±0.63	2.20±0.49	9.20±1.02
Apium graveolens	3	8.67±0.40	12.67±0.61	21.33±0.79
Pastinaca sativa	7	8.91±0.37	8.23±0.36	17.14±0.58
Daucus carota	7	6.20±0.31	7.26±0.25	13.46±0.36
Petroselinum crispum	5	14.72±1.01	12.48±0.26	27.20±1.27
Lactuca sativa	1	21.40±0.51	19.20±1.24	40.60±1.50
Brassica oleracea	2	1.10±0.28	2.80±0.51	3.90±0.64
Brassica oleracea var. *botrytis*	2	1.03±0.19	0.37±0.12	1.40±0.22
Cucumis sativus	3	11.70±0.78	14.00±2.07	25.07±2.28
Cucurbita pepo	1	42.20±0.80	51.40±4.76	93.60±4.48
Lycopersicon esculentum	13	5.91±0.23	4.85±0.53	10.75±0.61
Capsicum annuum	8	30.60±1.60	21.10±1.53	51.70±1.86
Mean at Vărăș ti	53	8.63±0.50	8.25±0.53	16.89±0.99

Restriction fragment length polymorphisms ($RFLP_s$) method has the advantage that it distinguishes species after extraction and purification of genomic DNA, restriction digestion and visualisation of banding patterns in gel electrophoresis. The 760 bp PCR product we obtained for the amplified ITS region with 18S and 26S primers. After digestion PCR products with the two restriction enzymes , *Meloidogyne hapla* isolate showed the following restriction patterns: 380 bp with DraI and 620, 140 bp with RsaI and for *Meloidogyne incognita* isolates: 220, 200, 180, 160 bp with DraI and 760 bp with RsaI (Figure 7).

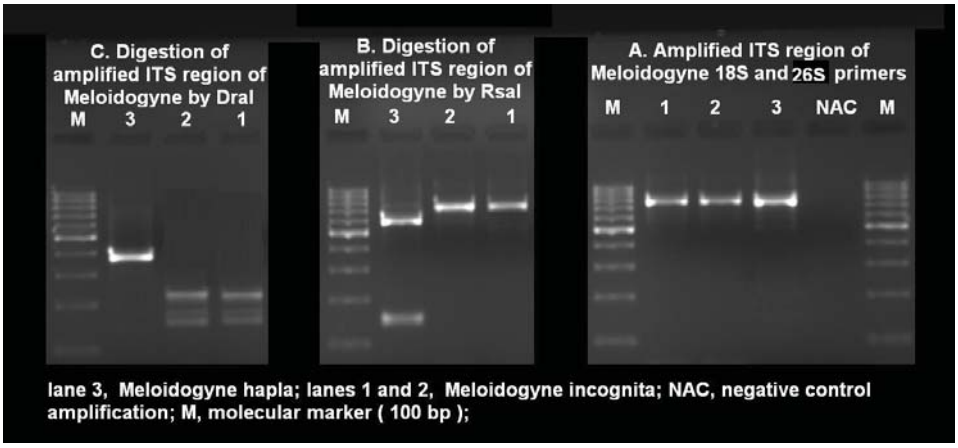

Figure 7. **A**, typical amplification by polymerase chain reaction (PCR) of 760 bp product from template of total DNA extracted from juveniles of *Meloidogyne hapla* and *Meloidogyne incognita* ; **B, C,** size of DNA fragments (bp) obtained after restriction enzyme digestion of the 760 bp internal transcribed spacer regions of *Meloidogyne hapla* and *Meloidogyne incognita* with RsaI and DraI.

CONCLUSIONS

The present study was elaborated to identify and quantify one of the most encountered species of polyphagous phyto-parasite nematodes in our country.

To better understand the economic impact of the attacks in case, a correct identification of RKN was necessary.

Both vegetables fields are favourable to the RKN development due to the soil, the temperature and the host-plants presence, as proved by the high global incidence which reaches 87.5%.

Until present, there was no aggressive attack to *Brassica oleracea* reported in the specialty literature from our country.

In this case, the significant density of nematodes in soil and roots, probably the susceptibility of the variety, leaded to an incidence of 100% to *Brassicaceae* family in Băleni.

The preponderance of the *Meloidogyne hapla* (50%) species was observed in Băleni unlike Vărăşti where the *Meloidogyne incognita* (53.33%) species predominates.

It is to remark that as far as *Lycopersycom esculentum* (culture of major economic importance) is concerned, the occurrence of the RKN was 100% which could be due, among other factors, to the use of Monkeymaker variety, sensible to RKN attack.

An index of the galls over 100/root was reported to the members of *Brassicaeae* family (Băleni).

The density of nematodes (in soil and embedded in the roots) is relatively close to each host plants.

The difference in terms of density among different plant species are due to climate, soil type, plant varieties, which favours the survival and multiplication of RKN.

Although molecular techniques are not readily available to every diagnostician, they complement and confirm the bio-morphometrical identification, thus increasing the reliability of diagnosis.

The combination of the conventional and molecular methods represents, from our point of view, a challenge for the discovery of other species of *Meloidogyne* parasitic in the vegetable areas from different areas of the country.

REFERENCES

Agrios G.N., 2005. Plant Pathology, 5th edn. Academic Press, USA.

Holbrook C.C., Knauft D.A., Dickson D.W., 1983. A Technique for Screening Peanut for Resistance to *Meloidogyne arenaria.* Plant Disease. 67 (9): 957-958.

Hussain M. A., Mukhtar T., Kayani M. Z., Aslam M. N.,. Haque M. I., 2012. A survey of okra (*Abelmoschus esculentus*) in the Punjab province of Pakistan for the determination of prevalence, incidence and severity of root-knot disease caused by *Meloidogyne spp*. Pakistan Journal of Botany, 44 (6): 2071-207.

Ibrahim S.K., Perry R.N., Burrows P.R., Hooper D.J., 1994. Differentiation of species and populations of *Ditylenchus angustus* using a Fragment of Ribosomal DNA. Journal of Nematology, 26:412–421.

Jepson S. B., 1987. Identification of root-knot nematodes (*Meloidogyne* species). Wallingford, C.A.B. International, London.

Karssen G., Moens M., 2013. Root-knot nematodes. In: Perry RN, Moens, M. Plant Nematology, 2nd edition. CAB International, Wallingford, UK, 59-90.

Manolache C., Pain S., Săvescu A., Bucș an I., Manolache F., Hrisafi C., 1949. Situaț ia dăunătorilor animali ai plantelor cultivate în anul 1947-1948. Seria Nouă (1): 11.

McKenry M. V., Roberts P.A., 1985. Phytonematology study guide. Univ. of California, Div. of Agri. and Natural Res. Pub.

Norton D.C., 1978. Ecology of plant parasitic nematodes.Wiley and Sons, New York.

Sharon E., Chet I., Viterbo A., Bar-Eyal M., Nagan H., Samuels G.J., Spiegel Y., 2007. Parasitism of *Trichoderma* on *Meloidogyne javanica* and role of the gelatinous matrix. European Journal of Plant Pathology,(118): 247-258.

Southey J.F., 1985. Laboratory methods for work with plant and soil nematodes. Her Majesty's stationary office, London.

Taylor A.L., Sasser J.N., 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Raleigh NC, USA.

Taylor D.P., Netscher C., 1974. Improved technique for preparing perineal patterns of *Meloidogyne* spp. Nematologica, 20(2): 268-269.

Viaene N., Moens, M., 2011. Root-knot nematodes in Europe. Nematology (13): 3-16.

Vrain T.C., Wakarchuk D.A., Levesque A.C., Hamilton R.I., 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology, 15: 563–573.

Zijlstra C., Lever A.E.M., Uenk B.J., Van Silf hout C.H., 1995. Differences between ITS regions of isolates of root-knot nematodes *Meloidogyne hapla* and *M. chitwoodi*. Phytopathology, 85: 1231-1237.

PM 7/41 (2) EPPO Standard . 2009, *Meloidogyne chitwoodi* and *Meloidogyne fallax*. Bulletin OEPP/EPPO 39, 5-17.


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