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TECHNQUE OF ROOT-KNOT NEMATODE, *Meloidogyne javanica*

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ABSTRACT

Bunt's technique (1975), is used for screening nematicides. A simple procedure has been developed using some modification that is suitable for rearing root-knot nematode with minimal chemicals, labors and greenhouse space as required. The different stages of nematode that used as initial inocula (egg-masses or second stage juveniles) succeeded in complement their life cycle and reproduction under irrigation with water or nutrient solution (0.8 gm ammonium sulfate 20.5%, 0.1 gm potassium sulfate 47%, and 0.6 gm calcium superphosphate 15% / liter of water).

Fertilizer treatment suppressed gall formation in case of inocula with egg-masses or juveniles whereas, such reduction was higher with plants inoculated with egg-masses than these inocula with juveniles. In contrast, fertilizers play an important role in increasing number of laying female. These findings are associated with increasing shoot moisture and decreasing root moisture. The results showed the highest juvenile yield number of egg-masses/ plant and percentage of egg-mass/gall ratio in case of inoclum with juveniles under fertilizer conditions. This work needs further investigations.

INTRODUCTION

Bioassay research on nematicides involves two components; the nematodes and poison, both of them must be standardized as possible, in order to obtain repeatable results. Generally, there are two sources for obtaining nematode individuals to be used in nematicidal bioassay researches under laboratory or greenhouse conditions: I- collection from field, II- rearing under greenhouse or laboratory conditions. Nematodes were responded as test animal to nematodes, depending on their genetic and environmental conditions through their life cycle i.e. temperature, food amount, food type and population density. Except parthenogenesis case standardize of genetic factors will be difficult, although it is easy to standardize environmental factors through rearing nematodes under controlled conditions. Reproduction in the root-knot nematode, *Meloidogyne chitwoodi* characterized by facultative meiotic parathenogensis (Triantaphllou 1985)

Many investigators reared different genera of plant parasitic nematodes under laboratory or greenhouse conditions (Seinhorst, 1959, El-Morshedy, 1985 and El-Kadi 1997). The development of rearing technique to produce root-knot nematode under standardized environmental factors would greatly facilitate the study of this nematode by providing continuous availability of nematode individuals under controlled conditions. Furthermore, this rearing method would be helpful in search for a control of root-knot nematode populations.

The purpose of this study is to describe a technique for rearing the root-knot nematode, by developing Bunt's technique (1975), designed to *screening nematicides.* Data *on nematode development and reproduction* were recorded to evaluate the level of success of this rearing method.

MATERIALS AND METHODS

Experimental design:

A population of *Meloidogyne javanica* was isolated from infected eggplants collected from fields near Khtatba, Menofya Governorate. Egg-masses were isolated from infected roots and suspended on Baermann pan, at room temperature25 ± 2°. The second stage juveniles (J2) were collected within 24 hours. Four-ml containing 375-second stage juveniles or two egg-masses of almost equal size were placed in 20-ml brown glass vials. About 10 gm of washed, sieved, and dried sand were added to each vial. A four-weeks old tomato seedling (c.v. Castle Rock) was planted in each vial.

Ten glasses inculcated with egg-masses were divided into 2 equal groups. The first one was irrigated as needed with 2 ml nutrient solution (0.8 gm ammonium sulfate 20.5%, 0.1 gm potassium sulfate 47%, and 0.6 gm calcium superphosphate 15% / liter of water) while the second one was only irrigated with water only (El-Kadi, 2002).

The previous procedure was carried out with ten glasses that inculcated with second stage juveniles. After 49 days the roots were washed free from sand. Data were recorded on plant growth, root galling as well as nematode development and reproduction as follows: Number of galls/ plant, number of egg-masses/plant, number of hatched juveniles/egg-mass, mean of (shoot–root) length and (fresh - dry) weight for shoot and root. Also rate of reproduction (Rf) percentage of egg-masses/galls ratio (EM/G), percentage of (root - shoot) moisture content and root/shoot ratio were calculated as follows: -

Rate of reproduction (Rf) = final population (Pf) / initial population (Pi)

$$\text{\% of egg-masses/galls ratio (EM/G)} = \frac{\text{No. of egg-masses/plant (EM/P)}}{\text{No of galls/plant (G/P)}} \times 100$$

$$\text{\% Root/Shoot ratio} = \frac{\text{Root}}{\text{Shoot}} \times 100$$

$$\text{\% of moisture content} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

All results were subjected to statistical analysis according to T. *student test (Gad and Weil, 1989). Significant difference were established at* $p<0.05$ levels.

RESULTS AND DISCUSSION

Data in Table (1) revealed that, tested individual stages of root-knot nematode, *M. javanica* which were used as initial inocula (egg-masses or juveniles) succeeded in complement their life cycle and reproduction through

irrigation with water or nutrient solution under suggested rearing technique conditions. According to the rate of reproduction (Rf), the initial inoculum was increased after 49 days to 10.3, 7.2, 2.28, and 5.8 times in case of plants inoculated with egg-masses and irrigated with water (EW), plants inoculated with egg-masses and irrigated with nutrient solution (EF), plants inoculated with juveniles and irrigated with water (JW) and plants inoculated with juveniles and irrigated with nutrient solution treatments, respectively.

Generally, the nutrient solution suppressed gall formation in case of inocula with egg-masses or juveniles compared with free nutrient solution treatments. These observations may be due to ammonium sulfate within nutrient solution, which inhibited development and reproduction of *Meloidogyne javanica*. These results agreement with these obtained by Spiegel *et al.* (1980), Dos Santos *et al.* (1981) and Fademi (1988).

Table 1: Root galling and reproduction of *Meloidogyne javanica* on tomato plants under improving rearing technique conditions.

Treatment	Mean No. of	Mean No. of	Mean No.of		Rate of		% of egg-masses/
	galls/plant	egg-masses	juveniles/		Reproduction (RF)		galls ratio
		/plant	plant	egg-masses	Juveniles	egg-masses	
Ew	119.4	20.6	1582.1	76.8	_	10.3	17.3
Ef	51.2	14.4	404.6	28.1	_	7.2	28.1
T-student	38.8	6.5	149.0	_	_	_	_
JW	98.4	14.2	815.04	57.4	2.28	_	14.4
JF	86.6	31.4	20506.7	65.5	5.8	_	36.2
T-student	7.9	19.5	165.6	_	_	_	_

JF- Plants inoculated with juveniles and irrigated with nutrient solution.

On the other hand, the numbers of galls on plants inoculated with egg-masses were less than those inoculated with juveniles in nutrient solution treatment. Numbers of gall were 51.2 and 86.6 with egg-masses and juvenile inocula in case of nutrient solution irrigation. This indication may be due to high sensitively of egg-masses to fertilizers. Also, fertilizers play an important role in increasing egg-masses /galls ratio compared with free fertilizer treatments. Percentages of egg-masses /galls ratio were 28.1 and 36.2 for Ef and Lf, whereas it was 17.3 and 14.4 in case of Ew and Lw. These results may be attributed to potassium sulfate within nutrient solution, which consider as favoring to growth and development of *Meloidogyne SP.* These results are in harmony with those reported by Oteifa (1953), Haque et al. (1974), Ismail and Saxena (1977) and El-Kadi (1990). Also, we could discuss the above result with data obtained by Smith *et al.* (1986), since, they indicated that supplemental phosphorus fertility stimulated egg production per female and increased the number of ovipositing when nematodes were added at planting but not when inoculation was delayed.

On the other hand, it is important to note that increasing in egg-mass /galls ratio were associated with decrease in number of juveniles/egg-mass with Ef and increase with Lf. The descending order of juvenile yield was Lf, Ew, Lw and Ef.

Data in Table (2) indicate that these are a significant difference between fertilized and non fertilized treatments with root length, shoot length and shoot fresh weight in case of egg-masses inocula, similar indication was observed in case shoot length with juvenile inoculum.

Table 2: *Meloidogyne javanica* on certain plant growth parameters of tomato under rearing technique conditions.

Treatment	Mean of root length cm	Mean of shoot length cm	Mean of root fresh weight gm	Mean of Shoot Fresh weight gm	Mean of Root dry weight gm	Mean of shoot dry weight gm	% Moisture content of shoot root	% root/ shoot rate
EW	9 2	20	0.691	0.861	0.07	0.114	89.8 86.7	61.4
EF	7.7	27	0.758	1.689	0.104	0.17	86.8 89.7	58.8
T-student	3.3	10.1	N S.	4.4	N.S.	N.S.	– –	–
JW	8.2	19.7	0 908	0.824	0.056	0.128	93.8 84.4	43.8
JF	8.4	17.9	0.741	1.23	0.087	0.087	88.4 93.8	100
T-student	N.S.	3.6	N.S.	N.S.	N.S.	N.S.	– –	

N.S.- Non significant
Ew- Plants inoculated with egg-masses and irrigated with water
EF- Plants inoculated with egg-masses and irrigated with nutrient solution.
JW- Plants inoculated with juveniles and irrigated with water.
JF- Plants inoculated with juveniles and irrigated with nutrient solution.

On the other hand, fertilizer recorded more increased moisture content of shoot than root with both inoculum methods. This finding was conjugated with increase in the egg-masses/galls ratio. The percentage of shoot and root moisture content were 89.9 and 86.8 in case of Ef, also it was 93.8 and 88.4 in case of Lf. The respective egg-masses /galls ratio were 28.1 and 36.2.

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طريقة تربية لنيماتودا تعقد الجذور (ميلدوجينا جافانيكا)

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في هذا الاختبار تم تطوير أسلوب بونت ١٩٧٥ لتقييم المبيدات لكي يصبح مناسبا لتربية نيماتودا تعقد الجذور بأقل قدر من الكيماويات والمجهود والمساحة ., وقد نجحت أفراد النيماتودا المستخدمة فى إجراء العدوى سواء كانت يرقات الطور الثانى أو أكياس البيض فى أن تكمل دورة حياتها وتتكاثر تحت ظروف ريها بمحلول مغذى (٨, جرام أمونيوم سلفات ٢٠,٥% +١, جرام بوتاسيوم سلفات ٤٧%+٦, جرام سوبر فوسفات الكالسيوم ١٥ % لكل لتر ماء) أو بالماء فقط .

وقد أدي التسميد إلى إنقاص عدد العقد المتكونة نتيجة العدوى بأكياس البيض أو اليرقات بينما كان هذا التأثير أوضح ما يكون فى حالة العدوى بالأكياس مقارنة بالعدوى باليرقات وقد كان للتسميد دور مهم فى زيادة كفاءة الإناث من حيث قدرتها على وضع البيض وقد أرتبط هذا المؤشر مع زيادة رطوبة الساق ونقص رطوبة الجذر. وقد سجل أكبر عدد من اليرقات المتحصل علية وكذلك أكبر عدد من الأكياس لكل نبات وأعلى نسبة لمعدل أكياس بيض/للعقد كنتيجة لأسلوب التربية المختبر فى حالة العدوى باليرقات المعاملة بالأسمدة.