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Evaluation of Four Tomato Genotypes Resistance to Root-Knot Nematode

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Abstract - ***The root-knot nematode incited by Meloidogyne incognita is one of the top diseases affecting tomato production worldwide. Nematicides can be effectively used to control the disease, but is costly and harmful to human life and environmental pollution. Alternatively resistant cultivars can be used inexpensive and eco-friendly. Resistance evaluation of five tomato cultivars (Red Rock, King Rock, Presto F1, Super Queen and Super Regina) were tested using randomized complete block design with five replications and three nematode inoculums (0, 500 and 1500 egg/second*** **juvenile** ***per pot). The statistical analysis gave significant differences among the genotypes, nematode densities level with their interactions. Among genotypes, Red Rock recorded significantly highest plant height (57.0) cm, Presto F1 (37.8) cm, King Rock (35.2) cm, Super Regina (30.1) cm and Super queen showed lowest (25.4) cm. As an alternative for managing the nematodes, further experiments are required in the field for applying plant resistances.***

Keywords: *Lycopersicon esculentum,* Nematode diseases, Disease Resistance, *Meloidogyne incognita.*

1. INTRODUCTION

There are several types of vegetables that have high rate of consumption by human around the world and Tomato (*Lycopersicon esculentum*) known to be included as one of the wide used vegetable worldwide [1]. The crop has developed into number of agricultural species and can adjust a variety of environmental conditions, different production procedure and food applicability. Tomato fruits in fresh or processed form have played a major role as an essential food commodity [2]. Root-knot nematodes (RKN) are the most important parasitic in the tropics. *Meloidogyne incognita* is one of the most widespread parasitic nematodes that infect plants in the tropical and subtropical regions [3, 4]. The genus *Meloidogyne* occurs as a pest on a very wide range of crops globally causing up to 5 % yield losses [5] and annual loss of $157 billion globally [6].

Meloidogyne spp. are able to destroy a big number of crops, especially veggies,in the tropical and sub-tropical and causes a destructive yield losses [7]. All control methods of RKNs can be categorized under one or more principles. Chemicals are used to eliminate nematodes but because of their cost and serious effects, nematicides are not attractive to farmers. Use of resistant cultivars might be the environmental procedure to manage RKNs. Resistance against *Meloidogyne* spp. has seen in several edible crops [8] but it is not applied frequently. The most essential evidence is the resistance against species (*arenaria, incognita,* and *javanica*) which returns to the *Meloidogyne* in *Mi*-gene bearing tomato type and are consumed extendedly. Though, resistant breaking numbers of *Meloidogyne incognita* and *Meloidogyne javanica* have been reported in Greece and Spain [9, 10] and this may decrease the current application. Resistance against some *Meloidogyne spp* was reported in prunes root stocks in France and Spain [11, 12]. Resistance against *M. javanica* was also found in plum and peach root stocks from France, Italy and Spain [13]. The aim of this research was to identify resistance against *M. incognita* among five different tomato cultivars.

2. MATERIALSAND METHODS

2.1. Experiment:

The research was set up in a farmer greenhouse located in village (Grdabor) near Piramagrun mountain 30 km west of Sulaimani Governorate, IKR/Iraq. After preparation of the research requirement, the experiment was set up on 15th July 2017.

2.2. Source of Nematodes:

Nematode inoculums were separated from the roots of infected tomatoes plant collected from infested fields of Sulaimani. Based on the morphological observations the nematodes species identified due to the perineal patterns images it was proved by one egg mass of a specific culture. From tomato root grown in greenhouse only 30 females were separated. The separation of the Perineal patterns were assessed according to [14].

2.3. Propagation of Tomato Genotype Seedlings:

Seeds of five tomato cultivars (Red Rock, King Rock, Presto F1, Super Queen and Super Regina) were separately planted in polystyrene seedling trays filled with sterilized soil. Seedlings were taken care of by daily watering and weekly fertilizing in the green house. Soil for the experiments pots (2 L) were composed of sand with a percentage of 65%, 25% clay and 10% O.M. Tomato seedlings (Five-week old) were transplanted into the pots.

2.4. Nematode Inoculums:

Infected tomato plant by southern root-knot nematodes was clearing from soil derbies. The galled root was slit into small pieces and shacked manually for 2-3 min in beaker contains 500 ml of 0.5% sodium hypochlorite (NaOCl) [15]. *M. incognita* eggs and juvenile's collections were standardized and concentrated according to [16]. Tomato seedlings with age of 5 weeks which 7 days passed their transplanting into the pots, were inoculated with 3 levels *M. incognita*, control (0 second juveniles /eggs pot), low (500 second juveniles/eggs pot) and high (1500 second juveniles /eggs pot). In a triangle shape 3 holes were dinged with size of 2cm from the roots.

2.5. Data Collection:

This experiments plant was harvested (55) days by the period of the nematode inoculation. The plant parts were separated from the roots by cutting off at the level of the ground then the vegetative parts of the plant were separated individually in a paper bags. By gently shaking the plants were washed and stored at a refrigerator at 4 ˚C. Plant height (PH) cm, fresh weight per plant (FWP) gm, dry weight per plant (DWP) gm and root gall index (GI) 0 – 5 scale; where 0=no knots (highly resistant), 1=1-2 knots (resistant), 2=3-10 knots (moderately resistant) , 3=11-30 knots (moderately susceptible), 4=31-100 knots (susceptible), and 5= over 100 knots/ plant root (highly susceptible). By using red coloring stains the system of the root were colored [18], Egg Mass Index (EMI) were checked by applying a (0 - 5) scale, where 0=no egg masses, 1= 1-2 egg masses, 2=3-10 egg masses, 3=11-30 egg masses, 4=31-100 egg masses, and 5= >100 egg mass / root system [17].

2.6. Statistical Analysis

Experiment was designed by randomized completely blocks design (RCBD) with five replications. All the data were analyzed by (ANOVA) by (JMP program from SAS version 7.0.1). Minimum variation LSD at (0.05) was used for comparing mean differences. The figures were drawing by Graph pad program version 7.0.

3. RESULTS

3.1. Identification:

The morphological characteristics of perineal pattern are similar to *M. incognita*as described by [19]. *Meloidogyne incognita* perineal pattern was oval shaped to round, striate usually wavy, in most cases with raised, squared, dorsal arch, lateral field weakly demarcated by forked striae (Fig 1).

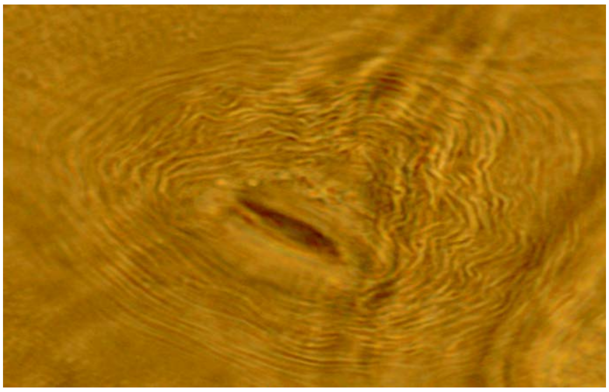

Figure 1:*M. incognita* detected photo.

3.2. Experiment:

ANOVA analysis indicates significant effect of tomato varieties on all parameters (P≤0.05). However, different nematode levels (N) affected PH, FWP, DWP, GI and EMI significantly. Additionally, there was a high significant influence between interaction nematode level (N) × tomato cultivars (C) for all parameters (Table 1).

Pooled data from appreciation marginal means (LSD) test, the PH was recorded relatively high for RR (577.000) cm and significantly reduced for SQ (25.400) cm. The FWP and DWP were statistically high in RR (50.133 and 6.100) gm respectively, the minimum decrease FWP was in SQ (29.600) gm, for DWP was recorded low in KR (3.806) gm. Among all tomato genotypes the GI and EMI were recorded the lowest in RR (1.200, 1.866), respectively. On the other hand, the GI was showed high in SR (3.333), then the EMI was showed high in SS and KR (3.333) (Table 2).

Data from nematodes densities level shown in Table 3. Inoculums densities of *Meloidogyne incognita* significantly affected morphological attribution of tomato plants. With an increase in the inoculums level significant decreases happened in the all parameters. The N1 was relatively recorded high measurements for all morphological attributes, except GI and EMI were uninfected. The infected plants decrease DWP and increase GI and EMI by increasing inoculums level respectively. However, there were no significant differences between inoculated treatments FWP and DWP.

Table 1: ANOVA for the effect of four genotypes and their 3 population levels of *Meloidogyne incognita* with interaction on PH, FWP, DWP, GI and EMI. (P≤0.05).

Parameters		**PH (cm)**		**FWP (gm)**		**DWP (gm)**		**Gall Index**		**Egg Mass Index**	
Source	***DF***	***F-Value***	***Sig.***	***F-Value***	***Sig.***	***F-Value***	***Sig.***	***F-Value***	***Sig.***	***F-Value***	***Sig.***
Cultivar (C)	4	276.6571	0.000	94.9955	0.000	76.0714	0.000	153.3636	0.000	146.5625	0.000
Nematode level (N)	2	68.4935	0.000	39.5495	0.000	77.6126	0.000	1054.162	0.000	1257.692	0.000
Cultivar * Nematode level(C*N)	8	4.4298	0.000	5.2642	0.000	8.7149	0.000	24.7027	0.000	48.4862	0.000

* LSD for treatments were Significant at (*P≤0.05*)

Table 2: Mean of selected genotypes for yield and RKN resistance parameter (Means ±SD).

Tomato Cultivars	PH (cm)	FWP (gm)	DWP (gm)	Gall Index	Egg Mass Index
Super Regina(SR)	30.133(d)± 6.534	38.466(b)±10.120	4.473(b)± 1.420	3.266(d)± 2.400	3.333(c)± 2.440
Super Queen (SQ)	25.400(e)±6.870	29.600(c)± 4.980	4.053(c)± 0.570	2.800(c)± 2.111	2.333(b)± 1.760
King Rock(KR)	35.266(c)± 8.300	36.933(b)± 4.680	3.806(c)± 0.720	3.333(d)± 2.440	3.333(c)± 2.440
Red Rock(RR)	57.000(a)±3.760	50.133(a)±2.670	6.100(a)± 0.870	1.200(a)± 1.010	1.866(a)± 0.740
Presto F1(PF)	37.800(b)± 2.910	31.000(c)± 3.800	3.373(d)± 0.360	2.466(b)± 1.880	2.133(b)± 1.680

*Datum are means of five replications where used to compare among plant genotypes. PH, FWP, DWP, GI: EMI.

*Datum means with equal letter none differ significant by LSD at ($P \leq 0.05$).

Table 3: Impact of inoculums of *Meloidogyne incognita* for yield and RKN resistance parameter (Means ±SD).

Nematode Inoculum	PH (cm)	FWP (gm)	DWP (gm)	Gall Index	Egg Mass Index
0 J2s (N1)	43.480(a)± 10.420	42.520(a)± 8.810	5.172(a)± 1.460	0.000(a)± 0.000	0.000(a)± 0.000
500 J2s (N2)	34.560(b)± 12.900	35.200(b)± 8.820	4.224(b)± 1.000	3.640(b)± 1.380	3.400(b)± 1.530
1500 J2s (N3)	33.320(b)± 11.510	33.960(b)± 8.780	3.688(c)± 0.800	4.200(c)± 1.120	3.800(c)± 1.380

*Datum are means of five replications where used to compare among plant genotypes. PH, FWP, DWP, GI and EMI.

*Datum means with equal letter none differ significant by LSD at ($P \leq 0.05$).

LSD tests showed that all the parameters were decreased by increasing nematode inoculums level. GI and EMI were not founded in all non-infested plants (Fig 2). The morphological features of tomato genotypes were found to be increasing at healthy plants compared to low level and high level of nematode inoculums. In SQ cultivar , the PH increases in non infested plants (34) cm following by low level (21.4) cm and high level (20.8) cm. FWP and DWP were recorded great at healthy plants (35.2) and (4.46) gm, low level (27.8) (3.96) gm and high level (25.8) (3.74) gm, respectively. Also GI and EMI were increasing by increasing level of inoculums, low level (3.8) (3.2) and high level (4.6) (3.8) respectively (Fig 2A). In RR genotype, the plants without nematode level, parameters such as PH, FWP and DWP recorded high values compared to other levels (60.8) cm , (52.8) gm, (7.12)gm respectively; the low level inoculums (PH: 57.2 cm, FWP: 49.4 gm, DWP: 6.06 gm, GI: 1.4 and EMI: 1.2), and high level (PH: 53 cm, FWP: 48.2 gm, DWP: 5.12, GI 2.2 and EMI: 1.4) (Fig 2B). The PH, FWP and DWP of SR cultivar healthy plants were showed high values (36.6) cm, (51) gm and (6.26) gm respectively. In addition, PH, FWP, DWP, GI and EMI by low level and high level application (27.2 cm, 31.6 gm, 3.9 gm, 4.8 and 5) (26.6 cm, 32.8 gm, 3.26 gm, 5 and 5) respectively (Fig 2C). In KR genotype, maximum value morphological attributes observed in non infected plants PH (46) cm, FWP (39.6) gm and DWP (4.48) gm. GI and EMI were recorded high value for both levels of nematode (5). PH (30.5) cm, FWP (36.2) gm and DWP (3.7) gm for low level. Although, high level inoculums recorded low value for PH (29) cm, FWP (35.4) gm and DWP (3.24) gm (Fig 2D). Finally PF genotype, the plant without nematode level, parameters such as PH, FWP and DWP recorded high (40) cm, (34.4) gm and (3.54) gm respectively. The low level inoculums showed low PH (36.2) cm, FWP (31) cm, DWP (3.5) gm, GI (3.2) and EMI (2.6), compared to other nematode inoculums level the high level of nematode recorder lowest PH (37.2) cm, FWP (26.6) gm, DWP (3.08) gm, also showed highest GI (4.2) and EMI (3.8) (Fig 2E).

4. DISCUSSION

The sustainable protected agricultural glasshouses face several nematodes challenge, particularly [20]. The RKN, *M. incognita*, in the tropical and subtropical origins a ruinous disorder of many crops owns a spreader host range (crops and weed) but not all are similarly good at sustaining nematode reproduction. Nematicides are usually expensive. They may lead to environmental pollution and their toxic residues may accumulate in edible plant products. Therefore, employ resistant genotype with methods of nematode control will shift the farmer from the concept of control to the concept of management. Results showed that number of galls indices were high on the root systems of the highly tolerance cultivar compared to susceptible and moderately susceptible. Minimum gall indices were recorded in the moderately resistant and resistant cultivars. Plant ability to inhibit growth or reformation of the nematode generally based on the resistance. Less and no gall indices indicated on highly resistant plant. Plant with less or mid ranges of resistibility response results a moderate reproductions amount however, a susceptible plant enable normal nematode growth to occur, and the occurrence of any disease that follows [21]. In the present experiment variability has been noticed in EMI and GI of *M. incognita* on five tomato genotypes. Susceptible cultivars showed great number of galls while lowest on moderately resistance and resistance genotypes.

When comparing the resistant cultivar and the susceptible one the development of the RKN eggs are poor [22]. However, the number of eggs is based on the quantity of the nematodes that is in the maturity reproductive stage and then the measurement of the resistance provided only once. The highly tolerance host plants permitted J2s to penetrate the roots, is at the development stage and formed eggs while, the plants that are resistible stopped their development for a while and as well stopped their reproductions. [23]. In the whole 5 genotypes of tomato that screened from the resistance to susceptible and based on the whole genotypes a huge differences revealed in their reaction to RKNs.

One cultivar (Tomato RR) was found to be resistance, and two others (SQ) (PF) were moderately resistance, while the last two (SR) (KR) were susceptible. In this study growth parameters given significant results with the amount of resistances against nematode. In tolerance plants galls are maintained by females of *Meloidogyne* and are with highly cellular modification. The highly tolerance cultivar showed maximum decrease in plant yield compared to moderately susceptible and susceptible cultivars [24]. Resistant genotypes showed better results concerning growth. The conclusion of the research work detect that *Meloidogyne incognita* quelled the tomato development with boost in inoculums and related decrease in the plant development. Harm reasoned by *Meloidogyne incognita* raised by raising inoculums thus there was raising harm among rising nematode densities and plant yield. Previous researches study have screened tomato cultivars to RKNs [25] and reported among 14 tomato genotypes only four of them resistant against *M. incognita*. [26] reported different response of cotton cultivars against RKNs and could help to minimize losses by the pest.[27] found okra genotypes resistant to tolerance in their response to *M. incognita*.

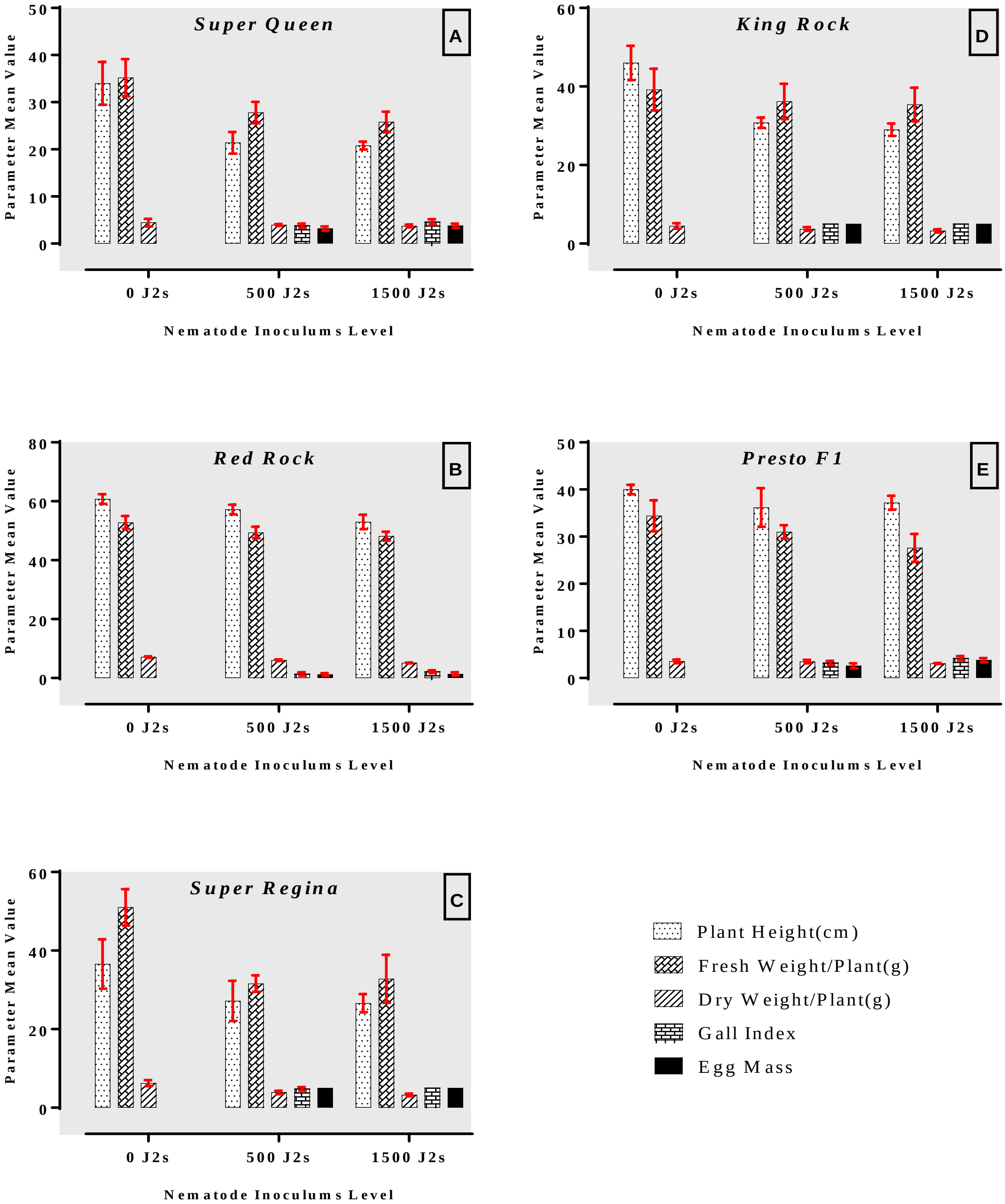

Figure 2: Effect of different nematode inoculums (0, 500, 1500 egg/ J2 per plant) on different tomato genotypes, plant morphological measurement contain PH cm, FWP gm, DWP gm. The figure showed nematode resistance parameter, Gall Index and Egg Mass Index (Mean Value ± Standard Error).

5. CONCLUSION

The findings of current study showed that there are statistically differences among tomato genotypes in their response to *Meloidogyne incognita*. RR genotype was found resistant. PF and SQ were found moderately resistant. The cultivation of moderately resistant and resistant genotypes would help to minimize the losses caused by RKNs. The approach will also help to diminish environmental pollution hazards and keep management processes more economical. Moreover, these genotypes could be used in breeding programs to introduce new resistant genotypes to RKNs.

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