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Evaluation of Two Vegetables Against *Meloidogyne incognita* Infection

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Abstract.- Chili (*Capsicum annuum* L.) and bell peppers (*Capsicum frutescens* L.) were evaluated for resistance to *Meloidogyne incognita* in a completely randomized design in a greenhouse trial. Fourteen chili and 5 bell pepper cultivars were inoculated with freshly hatched 5000 eggs of *M. incognita* and its response was evaluated 60-d post inoculation. Each plant was inoculated with 5,000 freshly hatched eggs of *M. incognita* and its response was evaluated 60-d post inoculation. Host suitability was categorized on the basis of reproduction factor [(Pf/Pi) where Pf =final nematode population/Pi=initial nematode population], root galling severity 0 to 9 scale, root galling and egg mass indices on a 0 to 5 scale, J2 per g of root and eggs per root system. The evaluation revealed C-19 chili genotype as non-host / resistance with zero, reproduction factor, root galling and egg mass indices, and root gall severity. Eight genotypes Sanam, Gola Peshawari, 15-2010, 11-2010, C-68, Tata Puri, C-302 and 28-2010 were categorized as good hosts / susceptible and the other five including 27-2010, C-73, C-72, 5-2010 and 18-2010 as fair host /moderately resistant ($5.0 \geq Pf/Pi > 1$). Two bell pepper genotypes Orible and F1 (pahuja seeds) were listed as poor hosts ($1 > Pf/Pi > 0$). Genotype Yolo Wonder and Capistrano graded as fair hosts /moderately resistant] ($5.0 \geq Pf/Pi > 1$) while genotype CDK-1000 as good hosts/susceptible ($Pf/Pi > 5.0$).

Keywords: Genotypes, host suitability, *Meloidogyne incognita*, reproduction factor.

INTRODUCTION

Presence of specific pathogens and parasites, including nematodes, can be responsible for extensive crop losses in tunnel-planted crops. Tunnel environments are akin to tropical paradise settings with plentiful moisture and warmth. The result is quicker development of plant roots, faster development of nematode generations and extended time for development of a greater number of generations each year. *Meloidogyne incognita* is recognized globally for its rapid rate of reproduction above 18^{O}C coupled with its pathogenic impact on a variety of crops. Their impact on the host can be extensive especially in tropical areas where environmental factors favor reproduction, survival, and dispersal of nematodes (Luc *et al*., 1990; Noe and Sikora, 1990). Only a few vegetable crops have nematode resistance. Crop rotation is seldom carried out in tunnel settings. Nematode control value of over-wintering does not occur, thus initial population levels are commonly high at time of planting and this applies to numerous nematode species. Root knot (*Meloidogyne* spp.), root lesion (*Pratylenchus* spp.), and burrowing (*Radopholus* spp.) nematodes are of significant economic importance in tropics (Anwar *et al*., 1992; Davide, 1988). It is time for greater attention to difficulties associated with tunnel farming in Pakistan.

Root knot nematode *M. incognita* is widespread and a major pathogen of vegetables in vegetable production regions of the world (Anwar *et al*., 2007, 2012; Ismail, *et al*., 2012; Naidu *et al*., 2006; Di Vito *et al.,* 1996; Sharma *et al*., 2004; Sant Yuji *et al*., 2004; Kamalwanshi *et al*., 2004; Piedra *et al*., 2006;).They cause damage by feeding and by inducing large galls or "knots" throughout the root system of infected plants, which can alter uptake of water and nutrients and interfere with translocation of photosynthates (Anwar, 1995; Davis *et al*., 2003; Williamson and Hussey, 1996). The degree of root galling generally depends on the magnitude of *Meloidogyne*-population density, host plant species and cultivar. Increase in the nematode population density results in an increase in the number of galls per root system. The nematode infection of roots increases the incidence and severity of fungal wilt diseases (Katsantonis *et al*., 2003), which can negatively influence yield (Orr *et al*., 1984). Severe nematode infections result in decreased yields of numerous crops and the quality of the marketable products is reduced by nematode infestations that

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cause tissue breakdown, deformation, or discoloration. Root systems may be deformed, and underground organs such as potato tubers and carrot tap roots may be damaged and become nmarketable (Roberts, 1987; Sikora and Fernandez, 1990). Yield losses to 19-vegetables due to nematodes can range from as little as 10% to over 75%, depending on soil type and prevailing weather conditions (Anwar and McKenry, 2012).

There are several pathogenic species of this nematode but the two most commonly found associated with tunnel-planted crops in the Punjab are *M. incognita* and *M. javanica*, which can attack numerous vegetable crops (Anwar *et al*., 2007; Anwar and Akhtar, 1992). Frequently, *Meloidogyne* interacts with other plant pathogens to form complexes particularly wilt diseases, in which the resulting disease is much more severe than that caused by either component alone (Katsantonis *et al*., 2003). These nematodes are particularly serious when high populations are allowed to build-up due to continuous replanting on the same site.

Understanding the host status of plant species to *M. incognita* will be useful in selection of crops to be used as rotation crops for the management of nematodes in tunnels, particularly in Pakistan, where effective nematicides are not easily available. The objective of this study was to determine the *M. incognita* reproduction rates on various cultivars of chili and bell pepper crops planted in the greenhouse at 25°C.

MATERIALS AND METHODS

Nematode inoculum

Meloidogyne incognita originally isolated from a single egg mass was increased on tomato cv. Money Maker in a green house. Eggs were collected from roots of tomato by placing in 800-ml sealed Manson glass jars with 1% NaOCl (Hussey and Barker, 1973), and shaken for 4 min at 200 cycles/min on a mechanical shaker (Eberbach Corporation, Ann Arbor, MI). This treatment was followed by a thorough rinse in tap water and eggs were counted at 40X magnification. Suspensions of eggs were stirred in tap water and counts adjusted to enable the desired inoculum density to be added per pot.

Evaluation of genotypes

Fourteen cultivars of chili (Table I) and 5 of bell pepper (Table II) were supplied by the Director of Vegetable Research Institute, Faisalabad.

Three week old plants of pepper genotype were transplanted into 13-cm dia. clay pots containing formalin sterilized sandy loam soil (70% sand, 22% silt, and 8% clay). Seven days after transplanting, the nematode suspension (5000 eggs) was poured into four holes about 3-cm deep around the base of each plant. The holes were then filled with soil and a little water was added to the pots. The check plants were inoculated with water only. There were three replicates of each genotype. Pots were arranged in a completely randomized design on a greenhouse bench. The experimental plants were irrigated after every two weeks with Hoagland's solution (Hoagland and Arnon, 1950).

Experiments were terminated 60 days after inoculation. Roots were washed free from soil, and root systems of the plants were stained with Phloxine B (Holbrook *et al*., 1983) and assessed for the presence of egg masses. Root systems were rated for galling and egg mass indices on a 0 to 5 scale (Quesenberry *et al*., 1989), where 0 = no galls or egg masses, 1 = 1 or 2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 > 100 galls or egg masses per root system. The root galling severity was assessed on 0 to 9; 1 = no galling 2 = <5% roots galled, 3=6-10%, 4=11-18%, 5=19-25%, 6=26-50%, 7=51-65% 8=66-75%, and 9=76-100% roots galled (Schoonhoven and Voysest, 1989). Eggs were extracted from each root system and counted to determine final population density for each plant. Nematode eggs from roots were extracted as above. Nematodes per gram of root were calculated to determine the reproductive ability of nematodes on each vegetable genotype. Host suitability was categorized as good, fair, poor and non-host on the basis of reproduction factor (Sasser *et al.*, 1984), root galling severity (Schoonhoven and Voysest, 1989), number of J2 per g of root as well as total number of eggs per root system (Zhang and Schmitt, 1994) and root galling indices ((Buena *et al*., 2007).

Data analysis

Data on nematodes were subjected to analysis of variance using SAS (SAS Institute, Cary, NC).

Means were separated by Duncan's multiple range test at $P = 0.05$ level.

RESULTS AND DISCUSSION

The *M. incognita* inoculum used was viable and reproductive as indicated by the development of root galling and presence of viable egg masses on roots of 13 of 14 chili genotypes, the exception being C-19. Nematodes also developed on roots of all 5 bell pepper genotypes tested (Tables I, II). Significant differences were observed among the genotypes evaluated in terms of root galling and egg mass indices, egg production, and reproduction factor. Galls and egg masses were evident on all good hosts. The host suitability of genotypes to nematodes has been designated on basis of reproduction factor (Sasser *et al*., 1984), root galling severity (Schoonhoven and Voysest, 1989), number of J2 per g of root (Zhang and Schmitt, 1994) and root gall indices ((Buena *et al*., 2007).

Host suitability based on reproduction factor and J2 per g of root

Meloidogyne incognita has a broad host range. It reproduced differentially on genotypes of both chilies and bell pepper. The only non-host genotype was C-19 with a reproductive factor [RF =Pi/ Pf] <1 (Table I). Eight genotypes (35.3%) were categorized as good hosts (susceptible) and included Sanam, Gola Peshawari, 15-2010, 11-2010, C-68, Tata Puri, C-302 and 28-2010. Chili pepper genotype C-19 was ranked as poor / non-host [resistant] (Pf/Pi = 0) while five others: 27-2010, C-73, C-72, 5-2010 and 18-2010 were ranked as fair hosts [moderately resistant] (if 5.0 ≥ Pf/Pi > 1). The average J2 population per g of root on these hosts ranged from zero in C-19 to 425 in C-302.

Two of five bell pepper genotypes seeds (F1) were listed as poor hosts (1 > Pf/Pi > 0) and. Genotype Yolo Wonder and Capistrano graded as fair hosts [moderately resistant] (if 5.0 ≥ Pf/Pi > 1) and genotype CDK-1000 as a good host (susceptible) when Pf/Pi > 5.0. The average J2 population per g of root on these five genotypes ranged from 2 in Orible to 250 in Capistrano (Table II). Roots of all genotypes that were categorized as poor or non-hosts had lowest RF, root galling and egg mass indices per root system suggesting that these genotypes are resistant. These findings agree with that reported by others for vegetables (Mani and Al-Hinai, 1996; Sharma *et al*., 2006; Brito *et al*., 2007).

Host suitability based on root galling severity

Root galling severity on a scale of 0 to 9 indicated infestation on roots of 14 chili genotypes by *M. incognita* that ranged from severe (8.5) on roots of 'Gola Peshawari' and to zero (0.0) on roots of C-19 with an average of 5.4 across all the chili genotypes (Table I). Root galling severity on roots of 5 bell pepper cultivars ranged from severe (7.07) on roots of CDK-1000 to light (1.0) on roots of seeds (F1) with an average of 3.0 on the three other genotypes (Table II). Our results concur with other reports on nematodes of vegetables (Potter and Olthof, 1993). Root galling severity is a measure of the size of the nematode population. High severity impacts foliar growth by inducing various physiological alterations in plant vital functions. The end result of poor foliage growth is ultimately reduced yield (Melakeberhan and Webster, 1993; Anwar, 1995)

Host suitability based on root galling and egg masses indices

The chili and bell pepper genotypes were classified into susceptible, moderately resistant, and resistant on root galling or/ egg mass indices on 0 to 3 scale (Buena *et al*., 2007). Eight chilies genotypes including Sanam, Gola Peshawari, 15-2010, C-68, Tata Puri, C-302, , C-72, and 28-2010 were ranked susceptible (root galling or/egg mass index > 3) while five others, namely11-2010, 27-2010, C-73, 5-2010 and 18-2010 were graded moderately resistant (root galling or egg mas indices = 1-3), and only one genotype (C-19) was found resistant (root galling or egg mass indices = 0) per root system. Yolo Wonder and CDK-1000 bell pepper genotypes were susceptible (root galling or/egg mass index > 3) while three others including Orible, Capistrano, and F1 (pahuja seeds) were resistant (root galling or egg mass indices = 1-3) to *M. incognita*. These findings are in line that has been reported for other plant species (Brito *et al*., 2003, 2007).

Table I.- **Host response of fourteen chili pepper genotypes to *Meloidogyne incognita* as measured by root galling, egg mass and reproduction factor in the greenhouses, 60-days after inoculation with an initial population density (Pi) of 5000 eggs per plant**

Chili genotypes	Eggs per root system	Indices		Pf/Pi*	Root galling severity [1-9]**	J2 per g	Host suitability*** based on	
		Eggs	Egg masses				Pf/Pi	Indices
Sanam	3870b	4	4	8.2	7.5	275	Good host	Susceptible
Gola Peshawari	19600c	4	4	9.8	8.0	340	Good host	Susceptible
C-19	0.00h	0	0	0.0	0.0	0	Non-host	Resistant
15-2010	981e	4	4	5.0	7.0	250	Good host	susceptible
11-2010	9760e	3	3	5.0	2.5	115	Good host	Moderately resistant
C-68	18760c	4	4	9.4	6.75	275	Good host	Susceptible
27-2010	4343g	3	3	2.1	3.75	276	Fair host	Moderately resistant
Tata Puri	40050a	5	5	20.0	8.0	405	Good host	Susceptible
C-302	34780b	5	5	17.3	7.75	425	Good host	Susceptible
C-73	4832g	3	3	2.4	4.0	267	Fair host	Moderately resistant
C-72	7172f	4	3	3.5	5.25	178	Fair host	Susceptible
28-2010	16980d	4	4	8.4	5.75	236	Good host	Susceptible
5-2010	3228g	2	3	1.6	4.25	179	Fair host	Moderately resistant
18-2010	3962g	3	3	1.9	4.5	202	Fair host	Moderately resistant

Data are mean of three replications; Means within a column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at $P = 0.05$.
*RF, Reproduction factor whereas Pf is final nematode population density divided by initial nematode population density.
**Rooting galling severity scale where: 1, no galling; 2, <5% roots galled; 3, 6-10%; 4, 11-18%; 5, 19-25%; 6, 26-50%; 7, 51-65%; 8, 66-75%; and 9, 76-100% roots galled (Schoonhoven and Voysest, 1989).
*** Host suitability Scale: where good host [susceptible] when Pf/Pi > 5.0, fair [moderately resistant] if 5.0 ≥ Pf/Pi > 1, poor if 1 > Pf/Pi > 0, and non-host [resistant] when Pf/Pi = 0 (Zhang and Schmitt, 1994).
***Host suitability Scale: S: susceptible, root galling or/ egg mass indices > 3 and; MR: moderately resistant, root galling or/ egg mass indices = 1-3; R: resistant, root galling or/ egg mass indices = 0 (Buena *et al*., 2007).

Table II.- **Host response of five bell pepper genotypes to *Meloidogyne incognita* as measured by root galling, egg mass and reproduction factor in the greenhouses, 60-days after inoculation with an initial population density (Pi) of 5000 eggs per plant.**

Bell pepper genotypes	Eggs per root system	Indices		Pf/Pi*	Root galling severity [1-9]*	J2 per g of roots	Host suitability*** based on	
		Eggs	Egg masses				Pf/Pi	Indices
Yolo Wonder	7536b	4	4	3.76	7.5	175	Fair host	Susceptible
SCDK-1000	14160a	4	4	7.07	8.0	240	Good host	Susceptible
Orible	1952d	3	2	0.97	1.1	2	Poor host	Moderately resistant
Capistrano	4495c	3	3	2.24	5.0	250	Fair host	Moderately resistant
F1(pahuja seeds)	4404c	2	1	1.0	1.5	3	Poor host	Resistant

Data are mean of three replications; Means within a column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at $P = 0.05$.
For other explanation see Table I.

Host suitability based on egg production

The lowest numbers of eggs on bell pepper roots occurred on Orible, Capistrano, and seeds (F1). These genotypes appeared to be less susceptible than Yolo Wonder were significantly ($P = 0.05$) less than that on the roots of CDK 1000, the most susceptible genotype of bell pepper (Table II). The roots of six chili genotypes 15-2010, 11-2010, 27-2010, C-73, C-72, and 18-2010 carried significantly fewer eggs and seemed to be less susceptible. Egg presence was intermediate on roots of Sanam, Gola Peshawari, C-68 and, 28-2010 genotypes which were all significantly (*P*= 0.05) fewer than that of Tata Puri, and C-302, the most susceptible chili genotypes (Table I). The roots of Genotype C-19 supported no reproduction of *M. incognita* and is designated as the only cultivar being resistant. The evaluation of host status on the criteria of egg production per root system has been used for ornamental and field crops (McSorley *et al*., 2004; Jenkins *et al*., 1995).

Root galling indices have been used to assess host status of annual and perennial crops to root-knot nematodes (Marull *et al*., 1994; Stirling and Cirami, 1998; Zhou *et al*., 2000). However, root galling is not a satisfactory indicator of the durability of root-knot nematode resistance (McClure *et al*., 1974; Hussey and Boerma, 1981; Reed and Schneider, 1992; Zhou *et al*., 2000; Anwar and McKenry, 2002). Reproductive factors (Pf/Pi) provide a better tool to assess the host status as they provide a basic measurement of the nematode's reproductive capabilities (Oostenbrink, 1966).

Only a small portion of commercially available genotypes were evaluated so there remains untapped potential to discover additional sources of resistance in chilies and bell pepper as improved root-knot nematode resistance is desired in the production of vegetables. Plant damage and nematode tolerance were not evaluated in this study, because noninoculated plants of each variety were not included. Evaluation of germplasm for tolerance is an important feature of nematode management programs (Young, 1998), and should be explored further with these two vegetables, in addition to studies of resistance.

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