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Resistance appraisal of *Solanum melongena* against *Meloidogyne incognita*

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Abstract

Screening of eggplant germplasm against root-knot nematode (*Meloidogyne incognita*) was evaluated to find the host status for five eggplant cultivars infected with *Meloidogyne incognita*. Seedlings of the cultivars Dilnasheen, Nirrala, VRIB-01, VRIB-04, and VRIBH-03 were transplanted into earthen pots containing sterilized soil. Repeat five times for each treatment. Thirty days after the transplantation, plants were inoculated with 2000 freshly hatched J2 of *M. incognita*. The nematode-inoculated plants were allowed to grow for 45 days and then harvested. Plants and roots were washed carefully in tap water. The root galling and egg mass indices were assessed on 0 to 5 scales and J2 root population was assessed. All the five cultivars were found susceptible based on the rate of reproduction of nematodes. Dilnasheen showed a maximum number of galls with the galling index of 5 and egg masses with egg mass index of 5 being most susceptible one while Nirrala was found to be less susceptible with a minimum number of galls with the galling index of 4 and egg masses with egg mass index of 4. None of the cultivars was found resistant to *M. incognita*.

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Introduction

Eggplant (*Solanum melongena* L.) is one of the most important Solanaceae crops and grows best in warm climates. 100grams of edible portion of eggplant contains 2.2grams of carbohydrates, 1.8grams of protein, and 2.2grams of fat, vitamin 520mg, iron 0.9mg, calcium 28mg, water 92.4ml, ash 1.3g and carotene 850mg (Meah, 2003). According to the FAO, the area cultivated under this crop was 9000 hectares with production of 89000 million tons in Pakistan which stands at 18th position in world ranking (FAO, 2016). The average yield of field and vegetable crops is very low because they are attacked by various yield reducing microorganisms such as fungi, bacteria, viruses nematodes and insects. Among these biological entities nematodes play a very important role in reducing the yield and doing immense damage of all field and vegetable crops. Root-knot nematode (RKN) disease comes first among the diseases and pests infecting eggplants in Kurdistan and Iraq (Stephen *et al.*, 2003 and Zwain, 2014). The nematode continues to damage eggplant at higher inoculum levels. Different symptoms are produced by RKN like the formation of root galls, stunted growth, and increased wilting with reduced uptake of water and nutrients and ultimately low yield from infected plants.

There are several plant parasitic nematodes that cause pathogenic problems on vegetables in Pakistan. RKNs are important agricultural pests worldwide, with the most common and damaging species being *Meloidogyne incognita* (Kofoid and White, 1919), *M. javanica* (Treub, 1885), *M. arenaria* (Neal, 1889) *Chitwoodi* (1949) and *M. hapla*. *M. incognita* is a major pest of vegetables all over the world particularly in Pakistan (Williamson and Hussey, 1996; Anwar *et al.*, 2007). RKN cause considerable losses to vegetable crops growing in sandy loam soils. Crop yield loss of 12.3% per annum in vegetables due to nematode infection has been recorded (Bridge and Page, 1980). Two RKN species including *M. incognita* and *M. javanica* have been reported to parasitize eggplant in the Punjab (Anwar *et al.*, 1991, 2007). The nematode infection also provides avenue to wilt pathogens to enter, which make the problem more complicated (Hutmacher and Davis, 2002).

Annual losses due to *Meloidogyne* spp. were 16.9% (Sasser, 1989) and losses up to 95% (Bourne *et al.*, 2004) and 24-38% (Sasser, 1979) yield losses were reported in tomato crops whereas on eggplant severe yield losses ranging between 17-20% (Sasser, 1979) and 30-60% (Lamberti, 1979) has been reported.

The approximate distribution of *Meloidogyne* spp. on the agricultural soils of Pakistan is *Meloidogyne incognita* 58%, *M. javanica* 31%, *M. arenaria* 8%, *M. hapla* 7% and other species are about 2% (Maqbool, 1986). Karssen and Hoenselaar (1998) described over 80 species of the genus, *Meloidogyne*. Castagnone-Sereno (2002) concluded that among them *M. incognita*, *M. Javanica*, *M. arenaria* and *M. hapla* were of outstanding economic importance because they were responsible for at least 90% of all damage caused by root-knot nematodes.

Sasser (1980) reported out of the 80 *Meloidogyne* species, *M. incognita* 47%; *M. javanica* 40%; *M. arenaria* 7% and *M. hapla* 6% are distributed and important. The warmer climates allow a longer growing season with more susceptible crops being grown per year and this together with shorter life cycle can lead to high nematode reproduction rates and severe crop losses. Sikora and Fernandez (2005) described that root-knot nematodes are notoriously difficult to manage because of their high reproductive potential.

Several control strategies such as biological control, rotation with non-host, sanitation and judicious use of nematicides have been reported to manage the root knot-nematodes (Whitehead, 1998). Biological control being the slowest method, chemical fumigants due to their high cost of application, hazardous to environment and increased regulation on use, and also biobased pesticides have limited nematotoxic activity as mature product is suitable for plants but do not have capability of controlling RKN because decomposition process eliminates the inhibitor so more management strategies for management of RKN (*M. incognita*) are currently being adopted (Nico *et al.*, 2004).

Resistance can be important for vegetables due to self-protection by resistance genes that impart

tolerance to nematode injury. Thus, these cultivars yield well under moderate to heavy infection without other management tactics. Moreover, cultivars with genes carrying moderate to high resistance blocked nematode reproduction significantly, resulting in decrease in nematode population levels in rhizosphere. This reduces the damage potential for the next crop in rotation. So the aim of this study was to find the resistance in eggplant cultivars as it is efficient and cost effective method to deal with nematode problems.

Materials and methods

During summer, 2018 a systematic survey was conducted to obtain a reliable estimate of infestation of root-knot nematode (RKN) on summer vegetables planted at grower's field located at the major vegetable production area of Lodhran. Root samples were carefully uprooted with a trowel, up to 15-20cm depth from the rhizosphere of plants together with approximately 1kg of adhering soil. Samples were put in polythene bags location, date and soil type etc. was recorded. A total of one hundred and Twenty samples were collected. These samples were transported in the laboratory of Plant Pathology Department, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur and were stored in a refrigerator at 5°C (40°F) until processed. To separate the nematodes from soil and roots, samples were processed to identify and assess the nematode population by counting them. Roots and soil were processed separately to assess the RKN population. Culture preparation was done on eggplant susceptible cv. Dilnasheen raised in sterilized sandy loam soil in earthen pots (20-cm dia.) which allowed growing for 30-days. Five eggplant cultivars including VRIBH-03, VRIB-01, VRIB-04, Nirrala, and Dilnasheen seedlings were raised in the greenhouse for thirty days in earthen pots (4 inches dia.) containing formalin sterilized soil. Each treatment was replicated five times. The plants were inoculated with 2000 freshly hatched J2 of *M. incognita* in 10-ml of water by pouring around each plant by making 3-4 holes and the pots were arranged in a greenhouse in a completely randomized design. Irrigation was done daily throughout the period of studies. Greenhouse

temperature ranged between 26-36°C. The growth of plants was observed regularly. After 45 days, plants were harvested from the pots and their roots were gently washed in tap water, dump-dried and weighed. Root systems of the plants were gently cut from the stem and the parameters including number of galls and egg masses per root system, galling index, egg mass index, root weight, final J2 population were recorded. Galling and egg mass indices were assessed on 0-5 scale, where 0 = no galls/ egg masses, 1 = 1-2 galls/ egg masses; 2 = 3-10 galls/ egg masses; 3 = 11-30 galls/ egg masses; 4 = 31-100 galls/ egg masses, and 5 = > 100 galls/ egg masses per root system (Quesenberry *et al.*, 1989). Roots were stained with Phloxine-B to assess the presence of egg masses (Holbrook, 1987). The rate of reproduction was calculated by dividing the final population by initial inoculum (2000 J2).

Statistical analysis

Data were subjected to ANOVA and significant differences among the means was partitioned by Duncan's multiple range test at $P=0.05$ (Steel *et al.*, 1997).

Results and discussion

Meloidogyne incognita produced a variable number of galls on roots of five eggplant cultivars. Data showing the Nematodes Population in 100cm³ of soil, Galling index, Total Samples, Infested Sample and Incidence recorded on five eggplant cultivars (Table 02). The comparison of all cultivars on the basis of these parameters indicated that none of the cultivar was resistant against, *M. incognita* infection. One hundred and twenty samples of soil and roots collected from 20 localities planted with summer vegetables including eggplant, okra and cucumber from District lodhran out of which 62 samples were found infected with RKN. The incidence ranged from 0 to 85.71% with an average of 47.1935% (Table-2). The galling index ranged from 0 to 5 with an average of 3. Both incidence and galling index varied from locality to locality. This variation might be due to the soil type. The species of RKN were identified; these species included *M. incognita* and *M. javanica*. The numbers of nematodes in roots were different in the locations (Table 02).

Table 1. Survey: Root -Knot Nematodes Associated with summer vegetable.

Vegetable Genotype	Localities Sampled
Eggplant (<i>Solanum melongena</i>)	Gogran, Jindomorr, Pipliwala,
	Qutubpur, Amir pur, Ghairbasti,
	Addashanal, Adam wahan, So chak,
	Cham morr, Molviwala, Tareen farm,
	Chak 384 WB, Super chowk,
	Kotlimorr, Duniapur, Jallaharain,
	Dhanot, Jeevanwala, Bastimalook
Okra (<i>Abelmoschus esculentus</i>)	Adam wahan, So chak, chammorr,
	Addashanal, Pipliwala, Molviwala
Cucumber (<i>Cucumis sativus</i>)	Addashanal, Molviwala, Pipliwala,
	Chak 384WB, Ghairbasti, Qutabpur

Table 2. Localities sampled for nematodes, nematode population in 20 g of root, Gallings index, total Samples, Infested samples and incidence.

Localities	Nematodes				
	Population in 100cm ³ of soil	Galling index*	Total Samples	Infested Sample	Incidence
Ghairbasti	215	4	6	3	50
Jeevanwala	237	4	5	3	60
Cham morr	152	3	8	3	37.5
Adam wahan	216	4	9	5	55.55
Treen farm	0	0	5	0	0
So chak	254	4	5	3	60
Duniapur	315	4	6	4	66.67
Pipliwala	319	4	4	3	75
Jallaharain	334	4	4	3	75
Dhanot	430	5	7	6	85.71
Kotlimorr	247	4	10	6	60
Super chok	144	3	8	3	37.5
Addashanal	122	3	6	2	33.33
Molviwala	0	0	4	0	0
Bastimalook	209	3	8	4	50
Qutabpur	133	3	9	3	33.33
Amirpur	0	0	6	0	0
Chak 384WB	186	2	7	2	28.57
Gogran	215	3	6	3	50
Jindomorr	419	5	7	6	85.71

The number of nematodes in soil was different in the locations. The data revealed that number of nematodes in soil were more in Dhanot as compared to other nineteen vegetable production areas. Two locations including Adam wahan and Gogran had the same number of nematodes in soil but were less from three locations Pipliwala, Jallaharain and Jindomorr. Fewer numbers of nematodes were observed in the

soil in Qutabpur, Addachanal, and Chak 384WB as compared to other locations. The infection was not found in three locations Tareen farm, Molviwala and Amir pur. Nematodes in soil samples of remaining 8 locations were also found but were less from Dhanot and Jallaharain and there were found great variation from location to location in different vegetable production areas in District Lodhran.

Reproduction of M. incognita on eggplant cultivars

The *M. incognita* produced galls on roots of all the five cultivars of eggplant (Table 03). The number of galls produced on roots of Dilnasheen was significantly ($P=0.05$) more as compared to the roots of all other four eggplant cultivars. The three cultivars VRIBH-03, VRIB-01 and VRIB-04 had the statistically same number of galls but less than Dilnasheen. Nirala was statistically least susceptible cultivar as compared to all the other four cultivars of eggplant as it had less number of galls. The *M. incognita* produced egg masses on roots of all vegetable genotypes (Table 03). The number of egg masses per root system produced on the roots of Dilnasheen were significantly ($P=0.05$) more as compared to other four eggplant cultivars. Nirala was found least susceptible as it had least number of egg masses on its root system. VRIB-04, VRIBH-03 and VRIB01 had statistically similar number of egg masses but less than Dilnasheen. Data regarding the root weight is presented in (Table 03). The eggplant cv. Dilnasheen had significantly ($P=0.05$) more root weight as compared to other four cultivars. VRIBH-03 cultivar stands second on the basis of root weight as it had weight higher than all other three cultivars but less than dilnasheen. The cultivars i.e.VRIBH-01 and VRIB-04 had statistically similar root weight. VRIB-01 and Nirala have less root weight respectively. Data regarding the rate of reproduction is presented in (table 03) the genotypes namely Dilnasheen had significantly ($P=0.05$) more rate of reproduction as compared to other four eggplant genotypes. Rate of reproduction on both VRIBH-03 and VRIB-01 was statistically similar. Nirala had least rate of reproduction as compare to all the other four genotypes. All the eggplant cultivars exhibited significant ($P=0.05$) response regarding the final population of RKN.

The Dilnasheen, had significantly ($P = 0.05$) more nematode population as compared to other four cultivars namely VRIBH-03, VRIB-01, VRIB-04 and Nirala. The less number of nematodes were from cultivar Nirala as compared to all the other four cultivars of eggplant.

All cultivars showed variability in their response to *M. incognita* infestation. However, *M. incognita* replicates on all cultivars and there was variability in pathogenicity, which might be due to presence of a nematode resistance gene (Hadisoeganda & Sasser, 1982; Roberts & Thomson, 1986). These genes made the plant less attractive for attacking nematodes. Different plant responses to nematode infection were observed. Compatible and incompatible reactions may be due to the presence of resistant genes that are activated as a result of nematode invasion and some

visible reactions can be observed in the plant cells (Williamson, 1999; Davis *et al.*, 2000; Williamson & Kumar, 2006). In the resistant plants nematodes fail to produce functional feeding sites in the host after invasion due to hypersensitive responses that leads in failure to develop subsequently as reproducing females (Williamson & Kumar, 2006). Roberts & May (1986) found greater number of females, galls and eggs per plant in susceptible cultivars inoculated with *M. incognita* as compared to moderately resistant cultivars. Two types of mechanisms for RKN resistance in plants have been reported including pre-infection resistance against RKN is due to presence of toxic or antagonistic chemicals in root tissue which prevent the entry of RKN in roots (Haynes & Jones, 1976; Bendezu & Starr, 2003) while in post infection resistance, nematodes penetrate roots but fail to develop.

Table 3. Greenhouse evaluation of five eggplant cultivars for resistance to root knot nematodes.

Eggplant Cultivars/lines	Root galls / root system	Galling index**	Egg masses / root system	Egg mass index**	Root weight (g)	Rate of Reproduction***	Final Population
VRIBH-03	118 ^b	5	140 ^b	5	18.94 ^b	3.65 ^c	7300 ^c
VRIB-01	109 ^b	5	131 ^b	5	16.53 ^c	3.25 ^d	6500 ^d
VRIB-04	124 ^{ab}	5	153 ^{ab}	5	18.59 ^b	4.45 ^b	8900 ^b
Nirala	45 ^c	4	51 ^c	4	10.15 ^d	2.15 ^e	4300 ^e
Dilnasheen	181 ^a	5	215 ^a	5	25.48 ^a	6.10 ^a	12200 ^a

**Gall and egg mass indices: 0-5 scale; where 0 = no galls or egg masses, 1 = 1-2 galls or egg-masses; 2 = 3-10 galls or egg masses; 3 = 11-30 galls or egg-masses; 4 = 31-100 galls or egg-masses, and 5 = > 100 galls or egg-masses per root system.

*** Rate of reproduction = pf/pi

It is often associated with an early hypersensitive reaction (HR) due to the death of the cell in root tissue around the nematode. This mechanism prevents the formation of a developed feeding site leading to resistance. Resistant tomato plants show typical HR upon a virulent RKN infection (Dropkin, 1969; Williamson, 1999). Bioteux & Charechar (1996) reported that resistant cultivars have gene of resistance in their gene pool that confers resistance to *M. incognita*. In the resistant roots, catalase activity is decreased as a result of RKN attack. There is a possible role of alkaloids or phenolics that may inhibit the synthesis of these enzymes and act as an elicitor of resistance in plant attacked by *Meloidogynes* species.

Although all the five eggplant cultivars were found to be susceptible but the infection was variable among the cultivars, which indicates that these cultivars vary genetically (Brian *et al.*, 2010). We have struggled to found the impervious germplasm of eggplant against *M. incognita* infection to obtain the resistant genes for transferring to commercial growing cultivars. This investigation has demonstrated that all the commercially grown cultivars are susceptible. On the other hand the root knot nematode has become one of the most damaging causes against efficient production of eggplant (Anwar *et al.*, 2007b).

This suggests that planting of non-host crops like cereals before planting the eggplants and growers should refrain from the planting of eggplants in the previously nematode infected fields.

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