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MANAGEMENT OF ROOT KNOT NEMATODE WITH MARIGOLD (*TAGETES ERECTA* L.*)* AND ANTAGONISTIC FUNGUS (*PAECILOMYCES LILACINUS* (THOM) SAMSON*)* IN TOMATO CROP

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ABSTRACT

In Pakistan, tomato (*Solanum lycopersicum*) has achieved second position among the most important vegetable crops and Root Knot Nematode (*Meloidogyne javanica*) caused economic losses in tomato production. Different quantity of marigold (*Tagetes erecta* L.) leaves along with different concentration of antagonistic fungus (*Paecilomyces lilacinus* (Thom) Samson) were used to control Root Knot Nematode on tomato plants in a glasshouse experiment. Significant increase in fresh and dry weight of root and shoot along with plant height was recorded with maximum quantity of marigold (10g/kg) and antagonistic fungus (10×10^3) while there was no significant increase in any parameter at low quantity (2, 4 and 6g/kg) and concentration (4×10^3 and 4×10^3). Maximum increase and decrease in number of galls, eggs masses, soil and root population, eggs per eggs mass and total population of nematode was recorded in the absence and presence of marigold and antagonistic fungus respectively. All parameters of host along with pathogen were statistically similar at intermediate doze of fungus and marigold.

Key-words: Nematode management, tomato, *Meloidogyne javanica*, *Paecilomyces lilacinus*, *Tagetes erecta*.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to family Solanaceae and it is widely cultivated in the tropical and temperate regions. In Pakistan, tomato is cultivated on 47.1 thousand hectares with an annual production of 502.3 thousand tones which is very low as compared to other growing tomato countries of the world (Anonymous, 2008). Biotic and a biotic factor play a vital role in the yield reduction of Solanaceous crop. Among biotic, fungus, bacteria (Ashraf *et at.*, 2012), virus (Abbas *et al*., 2012) and nematodes are responsible for this low yield. Root-knot nematodes (*Meloidogyne* spp) are economically important and caused 90 % yield losses (Castagnone-Sereno, 2002). In Pakistan, *M. incognita* (52%), *M. javanica* (31%), *M. arenaria* (8%) and *M. hapla* (7%) are reported due to favorable temperature, humidity and extensive cropping systems with maximum (100%) losses in Punjab (Khan, 2009). Synthetic nematicides are more effective to control nematodes than biological, physical, cropping system and organic soil amendments methods but nematicides have consequences to environment (Serfoji *et al*., 2010). The economic importance of nematode is increasing because the environmentalists strongly restrict the use of agrochemicals (nematicides) while crop rotation, use of resistant or tolerant varieties (Abbas and Hameed 2012) and biological agents are effective and environmental friendly tools to control the phytonematodes (Abid, 1996). Botanicals (plant based) pesticides have found as alternative to nematicides and are commercially available (Agnihotri *et al*., 1999). Marigold (*Tagetes erecta* L.) is used as inter cropping or in rotation for the management of root knot nematodes (Alexander and Waldenmaier, 2002). *Paecilomyces lilacinus* (Thom) Samson used as a antagonistic fungi for the management of root knot nematodes and root galling of *M. javanica* (Treub) Chitwood decreased when *P. lilacinus* was applied in a tomato (Khan *et al.*, 2006). The present study was conducted to evaluate the efficacy of *P lilacinus* and *T. erecta* for the control of *M javanica* and effect on host plant.

MATERIAL AND METHODS

Nematode inoculums: Tomato plants with conspicuous galls were uprooted (with soil) carefully from farmer's fields of Taxila and Rawalpindi district. The root-knot nematode (*M javanica)* was isolated from infected tomato roots and multiplied from a single egg mass on susceptible host tomato plant (Money maker) while confirmation was done by making perineal pattern (Taylor and Netscher, 1974). For collection of eggs, *M. incognita* infected roots were removed and washed while cut into small pieces (1-2 cm) and vigorously shaken in NaOCl (0.5%) for 5 min (Hussey and Barker, 1973). Juveniles were separated while eggs were collected on a 38 µm sieve and poured onto an extraction tray (Whitehead and Hemming, 1965). The freshly hatched second stage juveniles (J2s) were standardized and concentrated.

Efficacy of marigold and Antagonistic fungus: The sterilized wheat grains were inoculated with pure cultures of antagonistic fungus (*P. lilacinus*) and incubated at 25 ± 1°C for 15 days. The flasks were shaken for uniform colonization of the fungus and colony forming units per gram of the grains were counted using haemocytometer after making spore suspension. Sterilized soil (56% sand, 19% silt, 25% clay and pH 7.6) was sieved (3.5 mm) while crushed leaves of marigold (0, 2, 4, 6, 8 and 10gm/kg) were mixed in pots and allowed to decompose (15 days). An antagonistic fungus (*P. lilacinus*) was mixed in marigold amended soil at different concentrations and 3 week old tomato seedlings were transplanted in soil pots. Seven days after transplantation, the plants were inoculated with 2000 freshly hatched second stage juveniles of *M. javanica* and each treatment was replicated five times. The plants were placed in the glass house at 25 ± 1°C for 7 weeks. Growth variables, number of galls, egg masses, number of eggs per egg mass and reproduction factor was recorded after 7 weeks. The data were recorded on fresh and dry shoot and root weights, shoot and root lengths, number of galls, egg masses, and reproductive factor. For estimation of total nematode population, eggs were extracted from the roots of individual plants (Hussey and Barker, 1973) and the juveniles were extracted from the soil of each individual plant with the help of tray method (Whitehead and Hemming, 1965). The total number of eggs and nematodes in the soil constituted the total population and the reproductive factor (Rf) was calculated by dividing the final population (Pf) by the initial one (Pi) while the percent increases and reductions in these parameters were calculated (Puntener, 1981).

$$\% \text{ reduction/ increase} = \frac{\text{Value of the control plants - value of the inoculated plants}}{\text{Value of the control plants}} \times 100$$

The experiment was repeated twice and all the data were subjected to Analysis of Variance (ANOVA) using GenStat package 2009 and the means were compared by Student Newman Keuls Test at 5%.

RESULTS AND DISCUSSION

Root knot nematode were isolated for the roots of tomato plants and used for the experiment in the glass house (Fig. 1). Maximum increase in plant height, fresh and dry root and shoot weight was recorded when maximum quantity of marigold along with maximum concentration of fungus was applied while there was no significant increase in any parameter at low quantity and concentration. Plant hight did not increase when quantity of marigold was reduced and concentration of the fungus was increased gradually. Fungal concentrations of 8×10^3 and 10×10^3 were statistically similar with intermediated quantity of grated Marigold. The integration of marigold (2, 4, and 6 g) with four concentrations of the fungus gave similar increases in fresh root weight while the increases of fungus concentration with maximum quantity of marigold were found to be statistically similar. The concentration of the fungus at 4×10^3 cfus when added with minimum quantity of marigold resulted in the minimum increase in dry root weight while application of four concentrations of fungus with minimum quantity of marigold brought about statistically similar increase in dry root weights. Similarly no statistical difference in increase in fresh root weight was observed with 8×10^3 and 10×10^3 concentrations of the fungus. The increase in fresh shoot weight was statistically not different at four concentrations (Table 1). Maximum reduction in number of galls, eggs masses, soil and root population, eggs per eggs mass and total population was recorded with found with high quantity and concentration of marigold and fungus respectively while at low doze the minimum inhibition of every parameter was found. The first three treatments did not showed the maximum retardation and all were statistically same. Variance effect of integration of marigold along with fungus was observed on reproduction factor of nematode. Rate of nematode build up (Rf) was the maximum at lower concentrations of the plant and fungus while maximum reduction was observed where both were missed at highest rates (Table 2).

Fig. 1. Root Knot nematode infecting roots of tomato plant.

Table 1. Effect of Marigold and antagonistic fungi on tomato plants.

Marigold	***Paecilomyces lilacinus* (cfus)**				
gram	**4×10^3**	**6×10^3**	**8×10^3**	**10×10^3**	**Mean**
Tomato plant height					
2	6.09± 0.39a	6.16± 0.73a	7.54± 0.12a	8.13±0.83ab	6.98
4	8.63±0.57ab	9.9± 0.46abc	12.22±0.4bc	13.37±0.78c	11.03
6	10.5±0.69abc	16.76±0.45d	18.68±0.70def	20.34±1.07def	16.57
8	12.66±0.48bc	17.27±0.71de	18.63±0.58def	21.91±0.68ef	17.62
10	19.44±0.07def	19.61±1.79def	22.76±1.16f	29.54±0.95g	22.84
Mean	11.46	13.94	15.97	18.66	
Fresh root weight of tomato plant					
2	3.40 ±0.58a	4.60±0.84a	8.32±1.33a	5.84±0.71a	5.54
4	4.98 ±0.53a	6.41±0.55a	7.18±0.65a	7.46±1.16a	6.51
6	6.70 ±0.26a	7.40±0.80a	8.21±0.76a	8.74±0.63a	7.76
8	10.40±0.85a	17.21±0.91b	20.80±5.02b	27.45±1.48c	18.97
10	15.84±0.82b	31.83±1.41cd	31.80±0.92cd	36.66±1.11d	29.03
Mean	8.26	13.49	15.26	17.23	
Fresh shoot weight of tomato plant					
2	4.70±0.17a	5.90±1.15ab	7.62±0.69ab	6.50±1ab	6.18
4	5.86±0.17ab	7.32±0.66ab	7.50±0.39ab	6.80±1.09ab	6.87
6	6.60±0.07ab	7.60±0.76ab	8.70±0.76ab	9.20±1.24ab	8.02
8	5.80±0.06ab	9.40±1.13ab	10.41±1.80abc	11.20±0.82ac	9.20
10	12.41±0.83bd	15.20±0.56cde	16.20±2.44de	17.40±0.75e	15.30
Mean	7.07	9.08	10.08	10.22	
Dry root weight of tomato plant					
2	4.04±0.54a	4.32±0.45a	4.80±0.50a	5.60±0.65ab	4.69
4	4.78±0.47a	6.15±1.11abc	9.26±1.07bd	11.62±0.64d	7.95
6	6.13±0.80ab	10.60±0.50d	15.75±0.34e	16.76±0.73e	12.31
8	16.45±0.57e	17.01±0.25e	22.38±0.92f	23.26±1.09f	19.78
10	24.42±1.16f	25.39±0.91fg	26.05±1.14fg	28.56±0.68g	26.10
Mean	11.16	12.69	15.65	17.16	
Dry shoot weight of tomato plant					
2	5.00±0.38a	7.80±0.47abc	7.93±0.59abc	8.42±0.58a-d	7.29
4	6.00±0.99ab	7.90±0.67abc	8.70±0.94a-f	10.10±0.54a-g	8.18
6	8.66±0.21a-e	9.20±1.12a-g	10.71±0.67b-g	13.50±0.81d-j	10.52
8	11.42±0.78b-h	12.20±0.70c-i	16.22±0.84ij	16.24±0.89h-j	14.02
10	14.00±1.12e-j	18.00±1.14jk	21.00±1.70k	28.00±2.07l	20.25
Mean	9.02	11.02	12.91	15.25	

± are standard errors of mean, Means followed by the same letter are not significantly different at $P < 0.05$

Marigold at different rates (2, 4, 6, 8 and 10 g) and antagonistic fungus (*P. lilacinus*) with four concentrations for the control of *M. javanica* improved tomato plant growth. Integration of marigold with antagonistic fungus reduced number of galls, egg masses, eggs per egg mass, nematode populations and reproductive factors caused by *M. javanica. P. lilacinus* is known to reduce root galling and egg mass production of *Meloidogyne* spp. (Mousa *et al.,* 1995) and with marigold betters results were observed.

There are reports which showed similar results when nematicides were combined with *P. penetrans* and *Verticillium chlamydosporium* (Ehteshamul-Haque *et al.,* 1995) while reduction due to leaf extracts may be attributed to the water soluble compounds in the extracts which are highly toxic to nematodes (Siddiqui and Alam, 1989) and may increased host resistance (Alam *et al*., 1980). Marigold leaf extract contains considerably high concentrations of α-terthienyl (2, 2'- 2'-terthienyl), 5-(3-buten-1-ynyl)-2, 2'-bithienyl and thiophenes that exhibit high nematicidal activity against several plant parasitic nematodes by producing volatile fatty acids, phenols, and amino acids (Alam, 1976). Considerable amounts of phenols such as Hydroquinone, p-cresol, catechol, pyrogallol and gallic acid have been detected in castor, mahua, mustard, margosa and groundnut cakes and were found highly deleterious to nematodes both *in vitro* and *in vivo* (Alam *et al.,* 1979).

Table 2. Effect of marigold and antagonistic fungi on root knot nematode.

***Tagetes erecta*(g)**	***Paecilomyces lilacinus* (cfus)**				**Mean**
	4×10^3	**6×10^3**	**8×10^3**	**10×10^3**	
Number of galls on tomato caused by *M javanica*.					
2	4.84±0.54a	5.28±0.81a	6.22±0.46a	12.52±o.57cde	6.85
4	6.06±0.95a	9.04±0.69abc	7.2±0.33ab	14.7±0.58de	8.84
6	7.96±0.66abc	9.5±0.68abc	8.58±0.65abc	21.2±1.02f	9.05
8	8.54±0.41abc	11.54±0.64bcd	14.2±0.73de	23.5±o.86f	17.98
10	16.40±0.16e	16.94±0.23e	23.78±0.77f	29.50±1.00g	21.66
Mean	9.05	10.79	14.20	17.46	
Number of egg masses on Tomato caused by *M javanica*.					
2	4.6±0.50a	4.8±0.38a	5.9±0.41ab	7.4±0.63a-c	5.68
4	6.3±0.53ab	8.2±0.65a-d	8.78±0.70a-d	10.5±0.59a-e	8.45
6	8.46±0.46a-d	8.92±0.60a-d	9.62±1.05a-d	14.28±0.95c-g	10.32
8	12.78±0.81b-f	15.3±1.53d-g	19.2±1.25fg	20.46±1g	16.94
10	16.9±1.15efg	16.9±1.58e-g	20.78±0.86g	30.52±1.29h	21.28
Mean	9.81	10.82	12.86	16.63	
Number of eggs per eggs mass of *M javanica*.					
2	235.2±0.99c	229.8±0.65bc	227.6±0.81bc	218.4±0.95abc	227.8
4	227.4±1.07bc	228.8±0.93bc	223.2±0.77abc	216.2±0.96abc	223.9
6	221.2±1.02a-c	221.8±0.87abc	215.2±0.81abc	211.6±1.05abc	217.5
8	219.6±0.80abc	218.8±0.77abc	211.6±0.94abc	204.6±1.06ab	213.7
10	212.2±1.41abc	209.6±0.89ab	208.4±0.83ab	199.2±1.17a	207.4
Mean	223.1	221.8	217.2	210.0	
Soil population of *M javanica*.					
2	776.4±0.93m	727.6±1.09l	676±1.01k	594.8±0.75j	693.7
4	566±0.97j	471.2±1.71i	436.8±1.40g	402.8±1.51fgh	469.2
6	401.2±1.43fg	383.2±1.06f	326±3.30e	301.2±0.99e	352.9
8	299.2±1.40e	262.8±1.12d	220.8±1.17c	208±0.86c	247.7
10	190±0.91c	154±1.38b	129.6±1.19ab	104.8±1.11a	144.5
Mean	445.4	399.76	357.68	322.32	
Root population *M javanica*.					
2	6005±14.17f	4340±14.12e	3563±23.96de	3624±20.32de	4383
4	4205±15.72e	4060±18.69e	3344±22.90c-e	2829±14.49b-e	3610
6	2996±11.83b-e	2049±12.33a-c	1992±11.12a-c	1876±8.63abc	2228
8	2270±9.34a-d	1951±9.73abc	1884±11.78a-c	1437±8.96ab	1885
10	1613±9.35ab	1344±7.09ab	1102±5.66a	1072±6.3a	1283
Mean	3418	2749	2377	2167	4383
Total population of *M. javanica*.					
2	6681±13.44h	4800±15.03g	4743±13.72g	4443±17.62fg	5166.7
4	3862±23.09 e-g	3778±19.79e-g	3723±10.75e-g	3607±21.73d-g	3742.2
6	3046±8.34c-f	2959±14.36b-f	2485±10.87a-e	2422±8.45a-e	2728
8	2293±10.25a-e	2210±11.10a-e	2179±8.12a-e	2066±8.33a-d	2187
10	1746±6.60a-c	1645±8.46a-c	1323±5.10ab	1176±5.98a	1472.5
Mean	3067	3078.4	2890.6	2742.8	
Reproduction factor of *M javanica*.					
2	2.227±0.25h	1.6±0.27g	1.581±0.25g	1.481±0.32fg	1.721
4	1.287±0.42e-g	1.259±0.36e-g	1.241±0.20e-g	1.202±0.40d-g	1.328
6	1.015±0.15c-f	0.986±0.26b-f	0.828±0.20a-e	0.807±0.15a-e	0.903
8	0.764±0.19a-e	0.737±0.20a-e	0.726±0.15a-e	0.689±0.15a-d	0.612
10	0.582±0.12a-c	0.548±0.15a-c	0.441±0.99ab	0.392±0.11a	0.488
Mean	1.172	1.023	0.962	0.91	Mean

Significant different at $P < 0.05$, ± are standard errors of mean.

These toxic chemicals may cause immobilization, mortality, poor penetration, later retardation, feeding and reproduction of second stage juveniles while the unaffected females may develop normally but their egg laying potential may reduced by the application of leaf extracts (Bunt 1975). Each time when plants are watered, the soluble compounds of marigold and antagonistic fungi are drained into pore spaces where they kill the nematodes and thus bring about reduction in inoculum density and keep their populations below economic threshold levels (Siddiqui and Alam, 1989). Nematicides play a preventive role due to high solubility and the toxic chemicals which may reach into soil beyond the rhizosphere region of the plants and either kill or limit the mobility of nematodes populations but nematicides are not environmental friendly. Better growth of plants in the amended pots were appeared due to the management of nematode and organic amendments may change the physical structure and the fertility of soil resulting in increased tolerance of the plants to escape the nematode attack (Mahmood and Saxena, 1992). The decrease in number of the nematode and increase in growth of tomato proved the efficiency of marigold with antagonistic fungi and the results are much similar with previous study (Alam *et al*., 1979). This study reveals that marigold and antagonistic fungi reduced population of nematode while enhanced tomato growth and must used as a component of integrated nematode managements.

REFERENCES

Abbas, M.F., S. Hammed, A. Rauf, Q. Nosheen, A. Ghani, A. Qadir and S. Zakia (2012). Incidence of six viruses in potato growing areas of Pakistan. *Pak. J. Phytopath.,* 24 (1): 44-47.

Abbas, M.F. and S. Hameed (2012). Identification of disease free potato germplasm against potato viruses and PCR amplification of potato virus X. *Int. J. Biol. Biotech*., 9 (4): 335-339.

Abid, M. (1996). *Studies on the control of Root-knot Nematode (Meloidogyne incognita) with botanical toxicant.* Ph.D Thesis, University of Karachi, Karachi-75270, Pakistan. p.375.

Ashraf, A., A. Rauf, M.F. Abbas and R. Rehman (2012). Isolation and identification of *Verticillium dahliae* causes wilt on potato in Pakistan. *Pak. J. Phytopath.,* 24 (2): 112-116.

Alexander, S.A. and C.M. Waldenmaier (2002). Suppression of *Pratylenchus penetrans* populations in potato and tomato using African marigolds. *J. Nematol*., 34**:** 130-134.

Alam, M.M. (1976). *Organic amendments in relation to nematodes*. Ph.D. Thesis, Aligarh Muslim University, Aligarh. pp. 150.

Alam, M.M., A.M. Khan and S.K. Saxena (1979). Mechanism of control of plant parasitic nematodes as a result of the application of organic amendments to the soil. V. Role of phenolic compounds. *Ind. J. Nematol*., 9: 136-142.

Alam, M.M., M. Ahmad and A.M. Khan (1980). Effect of organic amendments on the growth and chemical composition of tomato, egg-plant and chilli and their susceptibility to attack *Meloidogyne incognita. Plant and Soil*, 57: 231-236.

Agnihotri, N.P., S. Walia and V.T. Gajbhyie (1999). Green pesticides, protection and safety evaluation. *Ind. Agri. Res. Inst*., New Delhi.

Bunt, J.A. (1975). Effect and mode of action of some systemic nematicides. *Meded. Landbhogesch. Wageningen* 75: 1-128.

Castagnone-Sereno, P. (2002). Genetic variability in parthenogenetic root-knot nematodes, *Meloidogyne* spp and ability to overcome plant resistance genes. *Nematol*., 4: 605-608.

Ehteshamul-Haque, S.M. Abid, V. Sultana, J. Ara and A. Ghaffar (1996). Use of organic amendments on the efficacy of biocontrol agents in the control of root-rot and root-knot disease complex of okra. *Nematol., Medit*., 24: 13-16.

Eisenback, J.D.H, J.N. Hirschmann, Sasser and A.C. Triantaphyllou (1981). *A more complete characterization of the four most common species of root-knot nematodes (Meliodogyne spp.) with pictorial Key*. IMP publication Raleigh, N.C.U.S.A

Hussey, R.S. and K.R. Barker (1973). A comparison of methods of collecting inocula of *Meloidogyne spp*., including a new technique. *Plant Disease Rept*. 57: 1025-1028.

Serfoji, P., S. Rajeshkumar and T. Selvaraj (2010). Management of root-knot nematode, Meloidogyne incognita on tomato cv Pusa Ruby. by using vermicompost, AM fungus, Glomus aggregatum and mycorrhiza helper bacterium, Bacillus coagulans. *Journal of Agri. Tech*., 6: 1.

Khan, A., K. L. Williams and H. K. M. K. Nevalainen (2006). Control of plant-parasitic nematodes by *Paecilomyces lilacinus* and *Monacrosporium lysipagum* in pot trials. *Biocontrol*, 15: 643–658.

Khan, A.S. (2009). *Screening of tomato cultivars against root knot nematodes and their biological management*. Ph. D. thesis in plant pathology, Agri. Uni. faislabad, Pakistan. pp. 51-52.

Mahmood, I. and S.K. Saxena (1992). Effect of green manuring with certain legumes on the control of plant parasitic nematodes. *Pak. J. Nematol*., 10: 139-142.

Mousa, E.M., A.M. Basiony and M.E. Mahdy (1995). Control *of Meloidogyne javanica* by *Verticillium chlamydosporium* on tomatoes. *Afro-Asian J. Nematol*., 5(1): 113-115.

Puntener, W. (1981). *Manual for Field Trials in Plant Protection*. Agricultural Division, Ciba Geigy Limited, Basle, Switzerland, 205pp.

Taylor, D.P. and C. Netscher (1974). An improved technique for preparing Perineal pattern of *Meloidogyne* species, *Nematol*., 20: 268-269.

Siddiqui, M.A. and M.M. Alam (1989). Effect of root-exudates of neem and Persian lilac on plant parasitic nematodes. *Anzeiger fur S'chadlingskunde Pflanzenschutz Urnweltschutz*, 62: 33-35.

Whitehead, A.G. and J.R. Hemming (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Ann. App. Bio*., 55: 25-38.

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