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ACTIVITY OF HUMIC ACID AGAINST ROOT KNOT NEMATODES ON TOMATO

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Abstract

This study was conducted to evaluate the efficiency of humic acid on root knot nematode in laboratory and under glasshouse conditions. Second stage juvenile (j2) were treated with 0.5, 1, 1.5, 2 and 2.5 ml of concentrated humic acid in petriplares. The soil around tomato plants in pots was treated with same above concentrations in glasshouse. Results showed that humic acid had significant lethal effect on j2 in laboratory test that increased with humic acid concentration. The highest effect 185.0 was obtained with 2.5 ml of concentrations humic acid compared with 35.0 in control. The application of humic acid in the soil around tomato plant root previously inoculated with root knot nematodes reduced significant the number of galls on the root and root galls index compared with untreated control plants. The lowest root gall index 1 was found on plant roots treated with 2.5 ml of concentrated humic acid compared with 4.6 in control. The reduction of galls was found associated with improvement of plant growth parameters. The highest increases in fresh and dry weights were manifested with 2.5 ml of humic acid that attained to 6.9 and 1.30 g. for shoot, 3.43 and 1.9 g. for root respectively compared with 3.6 and 0.603 g. for shoot, 1.33 and 0.52 g. for root respectively in control. The results suggest that soil treatment with humic acid offers significant nematode control and improves plant growth.

Keywords: *Meloidogyne* spp., Humic acid, Root knot nematodes.

Introduction

Tomato *Solanum Lycopersicum* family Solanacea is considered as one of the most important vegetable crops throughout the world. The areas cultivated with tomato, in the world, have attained to $5*10^6$ hectares with annual production of $161*10^6$ in 2012 (FAO, 2014). The annual production of tomato in Iraq was reported to be 286596 tons in 2016 (Statistic center, 2016; Al-Sandooq and Fattah, 2017). It has been reported that tomato fruit contains many nutrient elements, vitamins, and antioxidant compounds including Lycopene that reduce cancer infection (Giovannucci, 1999).Tomato plants are subjected to infection with many pathogens one of these *Meloidogyne* spp., the causal agents of root knot, were found to be the destructive that causing extensive root damage leading to serious economic losses in yields (Lamberti, 1997) (Al-Sandooq and Fattah, 2015). The earl infection with *meloidogyne* second stage juveniles cause seedling death, while the late infection cause reduction in plant growth and yield, as well as predisposes the plant to infection with other pathogens (Seenivasan and Senthilnadhan, 2017). More than too species of *Meloidogyne* were identified, among which *M. arenaria, M. incognita, M. javanica* and *M. hapla* were found to be the more important (Lamberti, 1997). The control of nematodes is restricted for long time on chemical nematicides, but due to excessive and misuses of nematicides, enormous problems for ecosystem, human and animal were created. So, the efforts were oriented to ward searching of alternative to nematicide, safe and effective to mange diseases caused by nematodes. As alternative to nematicide bio control agents such as growth promoting Rhizobacteria and fungi *Trichoderma viride* have been used to control nematodes. Humic acid, by product, produced during decomposition of organic matter, has been reported to improve growth and yield of many crops (Khattab *et al*., 2012; Khan *et al*., 2013). Other studies reported that humic acid effect eggs hatch and survival of *M. incognita* second stage juvenile In Vitro (Seenivasan and Senthilnathan, 2017).

The objective of this study is to evaluate the effect of humic acid on the reproduction of tomato root knot nematodes (*Meloidogyne* spp.) In Vitro and under glass house conditions.

Materials and Methods

Isolation of root nematodes

Tomato plants showing symptoms of growth reduction and wilting during hot days, suspected to be infected with *Meloidogyne* spp. were uprooted from tomato fields in Baghdad areas. The galled roots were cut into 2-3 cm. pieces and soaked in 1% sodium hypochlorite solution for 4-6 mints with moderate agitation. The suspension was passed through a series of 300, 150, 25 µm sieves. The eggs were washed with tap water to remove the traces of sodium hypochlorite and collected in glass cylinder of 150 ml, containing sterile water. The eggs suspension was maintained at 25 ± 2 °C for three days and the freshly hatched second stage juvenile (j2) were used next experiments.

Effect of humic acid on *Meloidogyne* spp. second stage juvenile (j2) survival under laboratory conditions

Five hundred j2 were added into 20 ml sterile water in sterile petriplates. Various concentrations of humic acid, 0.5, 1.0, 1.5, 2.0, 2.5 ml were separately added into each plate. Five hundred j2 in 20 ml of sterile distilled water served as control. Total j2 live and dead were counted after 72 hrs. under lighting microscope at 100x. The experiment was conducted in a completely randomized design with three replications.

Effect of humic acid on *Meloidogyne* spp. infestation on tomato under glasshouse conditions

One month old tomato seedlings, super marmande, were transplanted into 1 Kg plastic pots, filled with sterile mix soil, in glasshouse. Humic acid concentrations, 0.5, 1.0, 1.5, 2.0, 2.5 ml in 20 ml sterile distilled water were added separately into each plant after 3 days of transplanting. The plants were then inoculated with j2 (500 j2/plant), by pipetting in holes around the roots. The pots were arranged in a complete randomized design with three replications. The plants were carefully uprooted after 60 days of inoculation. The shoot portion was cut at the base of the stem and the total fresh and dry weights of shoot and root systems were recorded. The root system was washed in water and the root knot index was assessed according to 0-5 scale, where 0=healthy root, 1=1-2 knots/root, 2=3-10 knots/root, 3=11-30 knots/root, 4=31-100 knots/root, 5= more than 100 knots/root (Taylor and Sasser, 1978).

Results

Effect of humic acid on *Meloidogyne* spp. second stage juvenile (j2) survival under laboratory conditions:

The results showed that humic acid had significant lethal effect on *Meloidogyne spp.* j2 with all concentrations compared with control. The lethal effect was increased with humic acid concentration. The highest effect was found with 2.5 ml of humic acid (Table 1).

The dead numbers of j2 were found to be, 57, 84.6, 103.3, 128.3 and 185 with 0.5, 1.0, 1.5, 2.0, 2.5 ml of humic acid respectively compared with 35 in control. Many previous studies reported that humic acid inhibited egg hatching and exerted a lethal effect on j2 (Seenivason and Senthilnathan, 2017; Kesba *et al.*, 2008).

Table 1 : Effect of humic acid on second stage juvenile (j2) under laboratory conditions

Humic acid concentration/ml	Number dead j2
0.5	57^{e}
1.0	84.6^{d}
1.5	103.3^{c}
2.0	128.3^{b}
2.5	185.0^{a}
Control	35.0^{f}
LSD	10.86

Effect of humic acid on *Meloidogyne* spp. infestation on tomato under glasshouse conditions

Results of this experiment indicated that application of humic acid in the soil around tomato plant roots previously inoculated with j2 reduced significant the number of galls on the root and root gals index with all the concentration used compared with untreated control plants (Table 2). The lowest of root gall index (1) was found on plant roots treated with 2.5 ml humic acid compared with (4.6) in control. Several organic acid were found to have nematicidal potential against different nematode species including root knot nematodes (Zaki *et al*., 2004; Min *et al*., 2007; Thoden *et al*., 2011; Renco *et al*., 2012).

The reduction of galls, on plant roots treated with humic acid and infected with root knot nematodes was found associated with improvement of plant growth parameter as proved by significant increases in fresh and dry weights of shoot and root system (Table 3). The highest increase in fresh and dry weights were manifested with 2.5 ml of humic acid. That attempted to 6.9 and 1.30 g for shoot, 3.43 and 1.9 g for root respectively compared with 3.6 and 0.603 for shoot, 1.33 and 0.52 g for root respectively in non-treated control plant. Similar results about the effect of humic acid on plant growth parameters were reported by (Seenivason and Senthilnathan, 2017).

Table 2 : Effect of humic acid on *Meloidogyne* spp. on tomato under glasshouse conditions

Humic acid concentration/ml	Root- knot index
.5	3.3^{b}
1.0	3.0^{b}
1.5	2.3^{bc}
2.0	1.3^{c}
2.5	1.0^{c}
Control	4.6^{a}
LSD	1.109

Table 3 : Effect of humic acid on growth parameters of on tomato plants, infected with *Meloidogyne* spp. under glasshouse conditions

Humic acid Concentration /ml	Shoot weight /g		Root weight /g	
	Fresh	Dry	Fresh	Dry
0.5	4.3^{d}	0.773^{d}	1.63^{de}	0.72^{e}
1.0	5.0^{c}	0.790^{d}	2.00cd	0.94^{d}
1.5	5.4^{c}	0.963^{c}	2.30^{c}	1.50
2.0	6.06^{b}	1.203^{b}	2.86^{b}	1.73
2.5	6.9^{a}	1.306^{a}	3.43^{a}	1.90
Control	3.6^{e}	0.603^{e}	1.33^{e}	0.57
LSD	0.508	0.091	0.419	0.161

Discussion

The results of this study showed that the mobility of j2 was significant reduced by humic acid in laboratory experiment, and the reduction was increased with increasing humic concentrations. The activity of humic acid against nematodes could due to activity groups in humic acid that react with groupie in nematode leuding to restrict its mobility. It was reported that humic acid contain many active groups including hydroxyl, carboxyl, phenolic and carbamyl (chitwood, 2002). (Jothi *et al*., 2009) reported that humic acid at 0.4-1.0% caused 93%- 100% mortality of *M. incognita* juveniles in vitro. Treatment of pots soil with different concentrations of humic acid and plants insulated with root knot nematodes induced significant reduction in root infection, root gall formation and reproduction of nematode. The reduction of root knot nematodes on plant in soil treated through activation of plant defense mechanisms leading to synthesize bioactive compounds able to inactivate nematode development and reproduction. It was reported that plant possess various inducible defense mechanisms to protect themselves against pathogens attack. These defense mechanism can be induced by treating plants with various agents, natural and synthetic (Pieters *et al*., 2001; Walters, 2010). Organic acids may affect nematode reproduction by affecting the biochemical defense mechanisms of plant, by increasing proteins and fatty acids in root tissues that may involve in synthesizing bioactive compounds able to oppose nematode reproduction (Kesba *et al*., 2008). The suppression of root knot nematodes by humic acid was found associated with considerable increase in plant growth parameters as shown by significant increase in shoot and root, fresh and dry, weights. The improvement of plant growth can be arributed mainly to suppression of nematodes in addition to nutrit conal value of humic acid and improvement of nutrients uptake. Similar results about the activity of humic acid on root knot nematodes on different plant species were reported (Sarvansprya and Subramanian, 2017; Khan *et al.*, 2013; El-Nemr *et al.*, 2012). These results indicated that humic acid may be promising in root knot nematodes management on tomato and improve plant growth.

References

Al-Sandooq, Dhulfiqar, L.E. and Fattah, F.A. (2015). Effect of plant physiology age on induced systemic resistance in tomato agent root knot nematodes by chemical inducers. The Iraqi Journal of Agricultural Sciences, 46(2): 236-245.

Al-Sandooq, Dhulfiqar L.E. and Fattah, F.A. (2017). Induced systemic resistance in tomato to root knot Nematodes by *Beauveria bassiana* and a mixture of mycorrhizal fungi. Arab Journal of Plant Protection, 35(2): 78-83.

Chitwood, D.J. (2002). Phytochemical based strategies for nematode control. Ann Rev Phytopathology. 40: 221–249.

El-Nemr, M.A.; El-Desuki, M.; El-Bassiony, A.M. and Fawzy, Z.F. (2012). Response of growth and yield of cucumber plants (*Cucumis sativus* L.) to different foliar applications of humic acid and bio-stimulators. Aust. J. Basic Appl. Sci., 6: 630–637.

F.A.O. (2014). FAOSTAT domain: production. Statistics Division, FAO, Roma, Italy.

Giovannucci, E. (1999). Tomatoes, tomato-based products, lycopene and cancer: review of the epidemiological literature. Journal of the National Cancer Institute 91: 317-331.

Jothi, G.; Ramakrishnan, S.; Kumar, S.; Jonathan, E.I. and Senthilkumar, P. (2009). Effect of humic acid on hatching, longevity and mortality of *Meloidogyne incognita*. Indian J Nematol 39: 175–177.

Kesba, H.H. and Mona, E.M. Al-Shalaby (2008). Survival and reproduction of *Meloidogyne incognita* on tomato as affected by humic acid. Nematology. 10(2): 243-249.

Khan, A.; Khan, M.Z.; Hussain, F. and Akhtar, M.E. (2013). Effect of humic acid on the growth, yield, nutrient composition, photosynthetic pigment and total sugar contents of peas (*Pisum sativum*). J. Chem Soc. Pak., 35: 206–211.

Khattab, M.; Ayman, M.E. and Arafa, H. (2012). Effect of humic acid and amino acids on pomegranate trees under deficit Irrigation. I: Growth, Flowering and Fruiting. J Hort Sci Ornamental Plant, 4: 253–259.

Lamberti, F. (1997). Plant nematology in developing countries: Problems and progress. In plant nematode problems and their control in the near east region. FAO plant production and protection paper, 144.

Min, YY.; Sato, E.; Shirakashi, T.; Wada, S.; Toyota, K. and Watanabe, A. (2007). Suppressive effect of anaerobically digested slurry on the root-lesion nematode, *Pratylenchus penetrans* and its potential mechanisms. Jpn J Nematol. 37: 93–100.

Pieterse, C.M.J.; Johan, A.; Van Pelt; Saskia C.M. Van Wees, Jurriaan Ton, aren M. Léon-Kloosterziel, Joost J.B. Keurentjes, Bas W.M. Verhagen, Marga Knoester, Ientse Van der Sluis, Peter A.H.M.; Bakker, L.C.; Loon, V. (2001). Rhizbacteria-mediated induced stemic resistance: triggering, signaling, and expression. European Journal of plant pathology, 107: 51-61.

Renco, M.; Sasanelli, N.; Papajova, I. and Maistrello, L. (2012). Nematicidal effect of chestnut tannin solutions on the potato cyst nematode Globodera rostochiensis (Woll.) Barhens. Helminthologia. 49: 108–114.

Seenivasan, N. and Senthilathan, S. (2017). Effect of humic acid on *Meloidogyne incognita* (Kofoid & White) Chitwood infecting banana (Musa spp.). International Journal of pest Management, 1-9.

Statistic center (2016). Crops and vegetable production statistic agriculture. Directorate Iraqi council of ministers, Iraq.

Taylor, A.L. and Sasser, J.N. (1978). Biology, identification and control of root-knot nematodes. North Carolina State University Graphics 111.

Zaki, M.J.; Javad, S.; Abid, M.; Khan, H. and Moinuddin, M. (2004). Evaluation of some chemicals against root-knot nematode, *Meloidogyne incognita*. Int. J. Biol. Biotech. 1: 613– 618.

Thoden, T.C.; Korthals, G.W.; Termorshuizen, A.J. (2011). Organic amendments and their influences on plant-parasitic and free-living nematodes: a promising method for nematode management? Nematol. 13: 133–153.

Walters, D.R. (2010). Induced resistance: destined to remain on the sidelines of crop protection. Phytoparasitica, 38: 1-4.