Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(4): 1105-1108

Received: 10-05-2018
Accepted: 13-06-2018

**Jothi G**
Department of Plant Protection, ADAC&RI, Trichy, Tamil Nadu Agricultural University, Tamil Nadu, India

**Poornima K**
Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

**Correspondence**
**Poornima K**
Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

# Effect of potassium humate on hatching, mortality and plant infectivity of meloidogyne incognita in tomato

**Jothi G and Poornima K**

**Abstract**
The effect of humic acid on hatching, mortality, host plant infection and galling of root knot nematode, *Meloidogyne incognita* were investigated at the glass house, Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, India during June 2015. Different concentrations of humic acid (0.2% to 1%) were tested for their influence on egg hatching and juvenile mortality of *Meloidogyne incognita in vitro*. Humic acid at all the concentrations tried, caused significant inhibition in egg hatching and recorded cent percent inhibition of egg hatching at 1% concentration. Penetration of nematodes was delayed by 4 days after innoculation. The number of nematodes penetrated the roots were 5 % on the 4$^{th}$ day and gradually increased and was 15 % from the 8$^{th}$ day onwards. Percent $J_2$ mortality of *M. incognita* increasing with per cent concentration of humate showed positive relationship. It is optimized that humic acid at 0.4% is effective to cause higher juvenile mortality (93%) with comparatively shorter period of exposure (48h).

**Keywords:** Potassium humate, root knot nematode, mortality, root inhibition

## Introduction
The genus *Meloidogyne*, the root knot nematodes are among the most damaging nematodes in agriculture. It is a polyphagous plant parasitic nematode with a wide host range [1]. Nematicides are usually not available or not affordable in developing countries and the demand for alternative, safe and inexpensive natural nematicides has promoted the screening of plants for anti-helminthic activities [6].

Organic acids may affect nematode reproduction on their host plants by affecting the biochemical defense mechanisms of plants by increasing proteins and fatty acids in root tissues. Such an increase may be involved in synthesizing bioactive compounds able to oppose nematode development and reproduction [11]. Organic materials also have other beneficial effects, such as on soil nutrients and by improving soil condition and enhancing soil biological activity and general crop performance [17]. Humic acid supplemented with NPK was less effective in reducing nematode activity than humic acid supplemented with heavy metals, but it was superior in improving tomato growth [13].

Recently, the use of organic acids to plant growth and diminish nematode populations has been recommended. Humic substances contain a wide variety of molecular components. Some typical components are: polysaccharides; fatty acids; polypeptides; lignin; esters; phenols; ethers; carbonyls; quinones; lipids: peroxides; various combination of benzene, acetal, ketal, lactol, and furan ringed compounds; and aliphatic (carbon chains) compounds [14]. The oxidative degradation of some humic substances produces aliphatic, phenolic, and benzene carboxylic acids in addition to n alkanes and n fatty acids. The major phenolic acids released contain approximately 3 hydroxyl (OH) groups and between 1 and 5 carboxyl (COOH) groups [19]. Four organic acids ranked first in percentage increase values of total phenol which were amounted to 8.39, 6.36, 4.43 and 2.68% for salicylic, citric, oxalic and humic acids comparing to nematode alone, respectively [10].

Humic acid which is a powerful fungal stimulant also provides biological benefits in relation to nematode management. Potassium humate consist of humic acid (65%) and chemically reactive groups which influence the plant growth directly or indirectly [2]. Through the availability of material is in plenty, the knowledge on its beneficial effects is lacking. Hence, the objective of the present study was to assess the influence of potassium humate extract on

hatching, mortality plant infectivity of *M. incognita* with a goal of identifying novel leads for nematicides or for use as soil amendments.

## 2. Materials and Methods

Water-soluble potassium humate (65%) from Neyveli lignite corporation, Pvt, Limited commercially available was used for the study at the glass house, Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, India during June 2015.

### 2.1 Preparation of Humic acid Solution

Water soluble potassium humate (65%) from Neyveli lignite corporation, Pvt., Ltd which is commercially available were used for preparing the solution of different concentration of 1, 2, and 3 per cent.

### 2.2 Collection of test nematodes

The root knot nematode, *Meloidogyne incognita* population was obtained from naturally infected tomato plants from the pure culture maintained in the glasshouse of Department of Nematology, TNAU, Coimbatore.

### 2.3 Hatching bioassay:

Uniform sized egg masses were picked from the roots infected with *M. incognita* and transferred to different concentration of humic acid in cavity black. The egg masses placed in sterile distilled water served as control. All the treatments were replicated thrice and the number of hatched juveniles hatched out was counted at an interval of 24h for three days.

### 2.4 Juvenile mortality

One hundred $J_2$ of *Meloidogyne incognita* were suspended in 1 ml of distilled water and 5 ml of the above prepared solution were taken in a cavity block to test the mortality of juveniles. All the concentrations were replicated four times and the nematodes were kept under atmospheric temperature. After 24h incubation all the mobile and immobile $J_2$ were counted per cell with the aid of an inverted microscope at magnification 100 X. The ratio [(number of immobile nematodes/ number of total nematodes (mobile and immobile)] directly expressed the percentage of paralyzed nematodes per cell [4].

### 2.5 Inhibition of root infestation

One hundred fresh mobile *M. incognita* $J_2$ suspension in 1.5 ml of distilled water were inoculated to 2 weeks old tomato seedlings (Varity Co-2) transplanted in 500 cm polypropylene tumblers containing 100 gm sterile sand and closed with cotton at the bottom. The plants were uprooted at 24 h interval; their roots were washed free of soil under distilled water and stained with acid fuchsin [3]. Nematodes that had penetrated roots were stained red and were counted by examination of tomato roots under a binocular microscope at magnification 20 X. Percent invasion potential was estimated from the ratio of stained penetrated nematodes to total nematodes in the inoculums.

### 2.6 Statistical analysis

Three replicates were used for each experiment treatments. All data were subjected to a one way analysis of variance and means were compared with Newmwn Keuls multiple range test ($P< 0.05$) using super ANOVA.

## 3. Results

The effect of different concentration of humic acid on egg hatching is presented in Table 1. The hatching rate increased as the time increased. Hatching inhibition increased as the concentration and time increased. Highest inhibition of hatching over the control was recorded at 1% concentration after 48 h. significantly greater mortality occurred at 1% concentration. Exposure time also had significant influence on juvenile mortality ($P <0.05$).

Penetration of nematodes was delayed by 4 DAI. The number of nematodes penetrated the roots were 5 % on the 4$^{th}$ day and gradually increased and was 15 % from the 8$^{th}$ day onwards. There were different stages of nematodes in the root system. Inhibition of nematode penetration of roots improved plant growth compared to infested tomato roots.

## 4. Discussion

Results obtained from the present study suggest that humic acid have great potential in inhibition of egg hatching and juvenile viability of *M .incognita*. Seenivasan &Senthilnathan [21] recorded hatching of *M. incognita* eggs was inhibited 50%–100% following incubation in 0.08%-2.0% humic acid *in vitro*. Exposure of juveniles (J2) of *M. incognita* to different humic acid concentrations significantly affected the mobility of J2 *in vitro*. The percent immobility of J2 increased with concentrations of humic acid up to 0.08%). Humic acid had a toxic effect on *H. multicinctus*, at 2% caused 88.2% mortality of *H. multicinctus* after 24 h exposure and reached 100% mortality after 48 h exposure [20] which is on par with the present study. Four different concentrations of humic acid such as 0.1%, 0.2%, 0.3% and 0.4% were evaluated and achieved higher egg hatch inhibition at 0.4% [18].

Since humic acid is a derivative of organic substances, amino acids released during decomposition of organic amendments are toxic to nematodes [15]. It is suggested that the phytotoxicity is attributed due to the presence of allelochemicals especially the phenol compounds [22]. Digested poultry manure inhibited 90 % of nematode mortality due to humic acid contents [8] and detectable effect on egg hatching and juvenile mortality were observed [7] which is in accordance with the present study. Humic acid supplemented with micro-elements was significantly better than other products in reducing the number of surviving juveniles and achieving the highest percentage of nematode inhibition (49.2%), followed by the two concentrations of Vydate, which were not significantly different from one another. Humic acid applied at the rate of 2 and 4 g granules appeared to be more suppressive to nematode build up. Humic acid applied as liquid at 4 mL/plant diminished significantly the nematode build up than 2 mL/plant but not as much as granule application. [13]. *In vitro* studies, survival and hatching of *Aphelenchoides*, *Helicotylenchus* and *Meloidogyne hapla* were reduced when nematodes were treated with fulvic, humic, acetic, butyric, formic and propionic acid [9]. Plants treated with fertilizers containing organic acids [17] or humic acid [24] were found to be stronger and better. A benefit of humate due to their ability to complex metal ions and form aqueous complexes with micronutrients and also may form an enzymatically active complex, which are usually assigned to the metabolic activity of living microorganisms [23]. Many studies reported that humate preparations succeeded to increase the uptake of mineral elements [16], to promote the root length and to increase the fresh and dry weights of crop plants [5]. Reduction in nematode roots infection in all treatment especially in the treatment *Pseudomonas fluorescens* plus humate (HA1) which recorded 98 % [12] as per the results of the present study.

**Table 1:** *In vitro* studies on the effect of different doses of humic acid on egg hatching of root knot nematodes

| Treatments | Egg hatching at | | | |
|---|---|---|---|---|
| | 24h | 48h | 72h | 96h |
| T1 – 0.2% | 80.6 (1.90) | 94.3 (1.97) | 75.3 (1.87) | 9.3 (1.00) |
| T2 – 0.4% | 29.0 (1.45) | 35.3 (1.54) | 12.3 (1.30) | 8.6 (0.98) |
| T3 – 0.6% | 7.6 (0.86) | 14.6 (1.16) | 9.3 (0.99) | 5.0 (0.75) |
| T4 – 0.8% | 2.1 (1.31) | 21.0 (1.31) | 15.0 (1.19) | 4.6 (0.74) |
| T5 – 1.0% | 4.6 (0.64) | 7.0 (0.82) | 0 (0) | 0 (0) |
| T6 – control | 42.0 (1.16) | 64.0 (1.79) | 102.3 (2.0) | 119.6 (2.07) |
| SEd | 0.07 | 0.05 | 0.5 | 0.07 |
| CD (5%) | 0.16 | 0.12 | 0.12 | 0.15 |

Figures with in parentheses are log transformed values.

**Table 2:** *In vitro* studies on the effect of different doses of humic acid on juveniles of root knot nematode

| Treatments | Juveniles survived | | | |
|---|---|---|---|---|
| | 24h | 48h | 72h | 96h |
| T1 – 0.2% | 56.0 (1.74) | 15.6 (1.21) | 7.7 (0.91) | 56.0 (1.74) |
| T2 – 0.4% | 30.6 (1.49) | 7.0 (0.89) | 0.6 (0.15) | 30.6 (1.49) |
| T3 – 0.6% | 18.3 (1.27) | 12.6 (1.12) | 2.7 (0.45) | 18.3 (1.27) |
| T4 – 0.8% | 11.0 (1.08) | 8.6 (0.96) | 0 (0) | 11.0 (1.08) |
| T5 – 1.0% | 0 | 0 | 0 | 0 |
| T6 – control | 100 (2.0) | 100 (2.0) | 100 (2.0) | 100 (2.0) |
| SEd | 0.04 | 0.07 | 0.18 | 0.04 |
| CD (5%) | 0.09 | 0.17 | 0.40 | 0.09 |

Figures with in parentheses are log transformed values.

**Table 3:** *In vitro* studies on the effect of different doses of humic acid on Percent mortality of root knot nematode.

| Treatments | Percent mortality | | |
|---|---|---|---|
| | 24h | 48h | 72h |
| T1 – 0.2% | 44 | 85 | 92 |
| T2 – 0.4% | 70 | 93 | 99 |
| T3 – 0.6% | 82 | 88 | 97 |
| T4 – 0.8% | 89 | 92 | 100 |
| T5 – 1.0% | 100 | 100 | 100 |
| T6 – control | 0 | 0 | 0 |

## 5. Conclusion

From the present study, it can be concluded that the nematode can be well managed with few organic additives. With the changing public attitude towards environment pollution due to the use of chemical nematicides, use of Potassium humate too would be most efficient when used in combination with other management practices that are currently available. Organic additives would not only enhance soil fertility, but also would increase microbial diversity, decrease population densities of root-knot nematodes, and ultimately increase crop yields.

## 6. Acknowledgement

The senior author is thankful to the Department of Science and Technology, GOI, New Delhi, for providing necessary facilities and financial assistance in the form of sanctioned project.

## 7. References

1. Almeida, AMSFde, MSNde, Santos A, Reyan MF. Host status of selected plant species for *Meloidogyne megadrora*. Nematropica. 1997; 27:1-6.
2. Antilén M, Silva K, Acevedo S, Amiama F, Faúndez M, Knicker H *et al*. Characterization of humic acids extracted from biosolid amended soils. Journal of soil science and plant nutrition. 2014, 14. versión On-line ISSN 0718-9516
3. Byrd DW, Kivkpatrick T, Barker KR. An improved technique for clearing and staining plants tissue for detection of nematodes. Journal of Nematology. 1983; 15:142-143.
4. Chen SY, Dickson DW. A technique for determining live second-stage juveniles of *Heterodera glycines*. Journal of Nematology. Gainesville. 2000; 32(1):117-121,
5. Chen YM, De Nobili, Aviad T. Stimulatory effect of humic substances on plant growth. In "Soil organic matter in sustainable agriculture". (Eds F. Magdoff, R.R. Weil). Boca Raton, FL. 2004, 103-130.
6. Chitwood DJ. Naturally occurring nematicides. 1993, 300-315.
7. Dias CR, Ferraz S. Effect of bio digested fraction of chicken manure on hatching and mortality of *Heterodera glyceines* juveniles. Nematologia Brasileria. 2001; 25(2):99-101.
8. Dias CR, Riberro RCF, Ferraz S, Vida JB. Effect of bio digested cow manure on hatching of Meloidogyne incognita eggs.Nematologia Brasileria. 1999; 23(2):34-39.
9. Elmiligy IS, Norton DC. Survival and reproduction of some nematodes as affected by muck and organic acids. Journal of Nematology. 1973; 5:50-54
10. El-Sherif AG, Gad SB, Khalil AM, Mohamedy, Rabab HE. Impact of Four Organic Acids on Meloidogyne Incognita Infecting Tomato Plants under Greenhouse Conditions. Global Journal of Biology and Agricultural Health Sciences. 2015; 4(2):94-100
11. Farahat AA, Al-Sayed AA, Mahfoud NA. Compost and other organic and inorganic fertilizers in the scope of the root-knot nematode reproduction and control of Meloidogyne incognita infecting tomato. Egypt Journal of Agro-nematology. 2010; 9:18-29.
12. Hager I, Tolba, Magda N, Mohamed. Effect of Chitinase Producing Bacteria and Humate on Growth, Productivity and Root Knot Nematode Control of Flame Seedless Grapevines. Nature and Science. 2016; 14(6):1-14.
13. Hosn, Hamed Kesba Mona, Al-Shalaby EM. Survival and reproduction of Meloidogyne incognita on tomato as affected by humic acid. Nematology. 2008; 10(2):243-

249
14. Hussein Khaled, Hassan A Fawy. Effect of Different Levels of Humic Acids on the Nutrient Content, Plant Growth, and Soil Properties under Conditions of Salinity. Soil and Water Research. 2011; 6(1):21-29.
15. Khan AM, Alam MM, Ahmad R. Mechanism of control of plant parasitic nematodes as a result of the application of oil cakes to the soil. Indian Journal of Nematology. 1974; 4:93-96.
16. Mackowiak CL, Grossl PR, Bugbee BG. Beneficial effects of humic acid on micronutrient availability to wheat. Soil Science Society of America Journal. 2001; 56:1744-1750.
17. Mcbride RG, Mikkelsen RL, Barker KR. The role of low molecular weight organic acids from decomposing rye in inhibiting root-knot nematode populations in soil. Applied Soil Ecology. 2000; 15:243-25
18. Saravanapriya B, Subramanian S. Management of Meloidogyne incognita on tomato with humic acid and bioinoculants. Annals of Plant Protection Sciences. 2007; 15:195-197.
19. Schnitzer M. Significance of soil organic matter in soil formation, transport processes in soils and in the formation of soil structure. Soil Utilization and Soil Fertility. Humus Budget. 1992; 4(206):63-81.
20. Seenivasan N, Senthilnathan S. Effect of humic acid on *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956 infesting banana (*Musa* spp.) Fruits, International Journal of Tropical and Subtropical Horticulture. 2018; 73(1):22-30.
21. Seenivasan N, Senthilnathan S. Effect of humic acid on *Meloidogyne incognita* (Kofoid & White) Chitwood infecting banana (Musa spp.) International Journal for Pest Management. 2018; 64(2):110-118.
22. Shaukat SS, Siddiqui IA. Effect of some phenolic compounds on survival, infectivity and population density of Meloidogyne javanica in mungbean. Nematologica Meditirenia. 2001, 29 (In press).
23. Stevenson FJ. Humus chemistry: Genesis, Composition, Reaction, 2nd ed., John Wiley and Sons, New York, 1994, 496.
24. Zhang X, Schmidt RE. Hormone-containing products impact on antioxidant status of tall fescue and creeping bentgrass subjected to drought. Crop Science. 2000; 40:1344-1349.