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Original article

Effect of humic acid on *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956 infesting banana (*Musa* spp.)

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Summary

Introduction – **The spiral nematode *Helicotylenchus multicinctus* is a global pest on banana. Humic acid, a by-product produced during decomposition of various plant origin organic matters, has now been widely used to improve plant growth and yield. Developing an organic method with a rapid reduction in the nematode population levels and a plant growth promotion is a long term thrust in banana research. Our studies aimed to determine the effect of humic acid on *H. multicinctus* and banana growth.** *Materials and methods* – **Direct exposure of mixed life stages (J2, J3, J4 and adult) of *H. multicinctus* to humic acid at different concentrations (0.1, 0.3, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, 50.0, 75.0 and 100%) was studied *in vitro*. The effect of soil drenching with 1, 2, 5 and 10% humic acid concentrations on *H. multicinctus* infestation in *Musa acuminata* (AAA) cv. Grand Naine was experimented under glasshouse conditions. The biochemical changes in root enzyme activities and phenolic contents caused by humic acid in banana roots were measured in pot experiments.** *Results and discussion* – **Humic acid had a toxic effect on *H. multicinctus*. Humic acid at 2% caused 88.2% mortality of *H. multicinctus* after 24 h exposure and reached 100% mortality after 48 h exposure. Soil application of humic acid significantly reduced *H. multicinctus* population in soil (53.6 to 56.6%), root infestation (39.1–44.5%) and root damage (42.9–47.1%) on banana compared to the untreated control. Multiplication of *H. multicinctus* in banana was reduced 38.0–42.8% due to humic acid drenching. Humic acid treated plants were significantly taller, thicker and had a higher number of leaves, heavier pseudostem, longer and heavier roots than untreated plants. All the concentrations tested (1, 2, 5 and 10%) were equally effective in controlling *H. multicinctus* infestation and improving the banana growth. Soil treatment of humic acid was found to induce systemic resistance against *H. multicinctus* by triggering the activity of defense enzymes such as peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase and total phenol content in banana roots. The reasons for *H. multicinctus* suppression, banana growth improvement and the mode of action of humic acid are discussed.** *Conclusion* – **Soil drenching with 1–2% humic acid significantly controls nematodes and improves the growth of banana. These findings shall be confirmed and adapted to field conditions.**

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Significance of this study

What is already known on this subject?

- Eco-friendly management of the spiral nematode *Helicotylenchus multicinctus* is a worldwide challenging task in banana.
- Humic acid is a natural organic product having stimulating effects on crop plant growth.

What are the new findings?

- Humic acid showed direct and indirect antagonistic effects on *H. multicinctus* infection on banana crop plants in greenhouse conditions.

What is the expected impact on horticulture?

- This work established the potential application of humic acid to offset the damaging effects of *H. multicinctus* infection on banana plantations in addition to stimulating the plant growth. Since humic acid is a natural product, the promotion of humic acid in banana cropping would contribute to maintain soil health.

Keywords
India, banana, *Musa acuminate*, humic acid, spiral nematodes, pest management, biological control, systemic induced resistance

Résumé

Effet de l'acide humique pour contrôler *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956 en culture de banane (*Musa* spp.).

Introduction – **Le nématode spiralé *Helicotylenchus multicinctus* est un ravageur mondial du bananier. L'acide humique, un sous-produit issu de la décomposition de diverses matières organiques d'origine végétale, est actuellement largement utilisé pour améliorer la croissance et le rendement des plantes. Développer une méthode biologique permettant une réduction rapide des niveaux de population des nématodes et stimulant la croissance des plantes est un objectif à long terme en recherche sur bananiers. Notre étude a visé à déterminer l'effet de l'acide humique sur la croissance de *H. multicinctus* et du bananier.** *Matériel et méthodes* – **L'exposition directe de *H. multicinctus* (aux stades J2, J3, J4 et adulte) à l'acide humique à différentes concentrations**

(0,1, 0,3, 0,5, 1,0, 2,0, 5,0, 10,0, 25,0, 50,0, 75,0 et 100%) a été testée *in vitro*. L'effet du trempage du sol avec des concentrations d'acide humique de 1, 2, 5 et 10% sur l'infestation par *H. multicinctus* des plants de bananier *Musa acuminata* (AAA) cv. Grand Naine a été étudié sous serre. Les changements biochimiques racinaires d'activité enzymatique et de teneur en composés phénoliques causé par l'acide humique aux racines de bananier ont été mesurés dans des expériences en pots. *Résultats et discussion* **– L'acide humique a eu un effet toxique sur *H. multicinctus*. A une concentration de 2% l'acide humique a causé 88,2% de mortalité sur *H. multicinctus* après 24 h d'exposition et 100% de mortalité a été atteint après 48 h d'exposition. L'application d'acide humique a réduit de façon significative la population d'*H. multicinctus* dans le sol (53,6 à 56,6%), l'infestation des racines (39,1–44,5%) et les dommages radiculaires (42,9–47,1%) chez les bananiers comparativement au témoin non traité. La multiplication de *H. multicinctus* dans le bananier a été réduite de 38,0–42,8% par détrempage à l'acide humique. Les plantes traitées à l'acide humique étaient significativement plus grandes, plus larges et présentaient un nombre plus élevé de feuilles, un pseudotronc plus lourd, des racines plus longues et plus lourdes que les plantes non traitées. Toutes les concentrations testées (1, 2, 5 et 10%) ont été similairement efficaces pour lutter contre l'infestation par *H. multicinctus* et améliorer la croissance du bananier. Le traitement du sol par l'acide humique semble avoir induit une résistance systémique contre *H. multicinctus* en déclenchant l'activité d'enzymes de défense telles que la peroxydase, la polyphénol oxydase et la phenylalanine ammonia lyase et en augmentant la teneur en composés phénoliques totaux des racines de bananier. Les raisons de la suppression de *H. multicinctus*, de l'amélioration de la croissance des bananiers et du mode d'action de l'acide humique sont discutées.** *Conclusion* **– Le trempage du sol avec 1–2% d'acide humique permet de contrôler de manière significative les nématodes et d'améliorer la croissance des bananiers. Ces résultats doivent être confirmés et adaptés aux conditions de plein champ.**

Mots-clés
Inde, bananier, *Musa acuminata*, acide humique, nématodes spiralés, gestion des ravageurs, lutte biologique, résistance systémique induite

Introduction

Banana (*Musa* spp.), a globally consumed fruit and the fourth important food crop after rice, wheat and maize in the world, provides livelihood and nutritional security to millions of people. The fruits are rich in carbohydrate, potassium, riboflavin, niacin, ascorbic acid, calcium, magnesium and phosphorus. Banana is grown in 150 countries on an area of 4.84 Mha producing 95.5 Mt (Singh, 2010). Among the pests and diseases affecting the productivity, nematodes are considered to cause devastating effect on growth causing extensive root damage resulting in serious yield losses. Crop losses by nematodes of banana are very high, with an average annual yield loss of 20% worldwide (Sasser and Freckman, 1987).

The burrowing nematode (*Radopholus similis* Cobb), root-lesion nematode (*Pratylenchus coffea* Goodey), spiral nematode (*Helicotylenchus multicinctus* (Cobb) Golden.) and root-knot nematode (*Meloidogyne incognita* Kofoid and White) are reported as destructive nematode pests on banana. Among them, *H. multicinctus* has been reported to infest on all varieties of banana in tropical and sub-tropical countries such as Fiji, Australia, Hawaii, Israel, Ivory Coast, Cuba, Dominican Republic, Mexico, Nicaragua, Peru, Columbia, Pakistan and India (McSorley and Parrado, 1986). It is an endo-parasite in banana, completes its life cycle within the root cortex, produces shallow, superficial lesions in banana roots which resemble like eruptions. Such lesions cause extensive root necrosis, die back of root eventually leading the plant to debilitation (Zuckerman and Stritch-Harari, 1963). Due to nematode feeding, the parenchyma cells were affected by rupturing of cell wall, depletion of cytoplasm, enlargement of nucleus, cell discoloration and finally necrosis. *H. multicinctus* infected banana plantations often express stunting, root decay and poor yield (Blake, 1966). It is responsible for a 33% reduction of fruit yield (Selvaraj *et al*., 2014).

The control of this nematode largely depends on the repeated use of carbofuran, a carbamate nematicide that maintains yields 30% greater than in untreated plantations (Seenivasan, 2017). However, the continuous use of chemical nematicides leads to soil and ground water pollution, deleterious effect on non-target organisms, toxicity to applicators and making the *H. multicinctus* population resistance to nematicides. Use of ecologically safe and inexpensive natural bio-products having nematicidal properties on target nematodes is one approach to address the ill effects of chemical nematicides. Farmers are applying organic materials to soil for centuries to improve plant growth and yield. The concept of organic soil amendments has also been found to be effective in the suppression of nematodes in many crops (Seenivasan *et al*., 2013; Seenivasan, 2010). Reductions of *R. similis*, *P. coffeae, H. multicinctus* and *M. incognita* populations occur after the addition of various organic materials such as leaf extracts, plant residues, oil cakes and distillery sludge to soil in banana (Seenivasan, 2017; Seenivasan *et al*., 2013; Youssef and El-Nagdi, 2010). Organic amendments while decomposition can release nitrogen compounds, organic acids, or other compounds that had adverse effects on nematodes (Oka, 2010; Thoden *et al*., 2011). Humic acid, a byproduct produced during decomposition of various plant origin organic matters, has now been widely reported to improve the growth and yield of many crops (El-Nemr *et al*., 2012; Khattab *et al*., 2012). It is extracted from lignite or low rank coals. Humic acid typically contains heterocyclic compounds with carboxylic, phenolic, alcoholic hydroxyl and carbonyl functional groups (Elmiligy and Norton, 1973). Because of the nature of their functional groups, it is possible that humic acids may have a detrimental effect on *H. multicinctus*. Developing an organic method with a rapid reduction in nematode population levels and growth promotion benefit is a long-term thrust in banana research. Hence, the present investigation was undertaken to determine the lethal effect of humic acid on *H. multicinctus* and to study the effect of humic acid on banana challenge inoculated with *H. multicinctus*.

Materials and methods

Helicotylenchus multicinctus inoculum

A population of *Helicotylenchus multicinctus* used for *in vitro* and glasshouse studies was originally isolated from the roots of *Musa* × *paradisiaca* (AAB) cv. Nendran grown in the Orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The infested roots were washed in water, cut into small bits, homogenized in blender and processed by modified Baermann funnel technique (Schindler, 1961). Mixed life stages of *H. multicinctus* were recovered and further multiplied on *Musa acuminata* (AAA) cv. Grand Naine cultivated in 15-cm diameter pots (5 kg capacity) containing sterile red earth:sand:farmyard manure (2:2:1) mixture in the glasshouse at 28 ± 4 °C. Ninety days after nematode inoculation, the *H. multicinctus* populations multiplied as pure cultures were used as inocula for *in vitro* and glasshouse studies. The mixed life stages of J2, J3, J4 and adult were used as inocula in all the experiments.

Humic acid

The humic acid (potassium humate) used in this study was obtained from M/s. Neyveli Lignite Corporation Limited, Neyveli, Tamil Nadu, India. It is a water-soluble dark brown liquid formulation with the specific gravity of 1.00–1.01 and viscosity of 2.00–2.14 $mm^2\ s^{-1}$. The humic acid content was 2–4% with pH of 8.0–9.5.

In vitro studies

To determine the effect of various concentrations of humic acid (0.1, 0.3, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, 50.0, 75.0 and 100%) on mortality of *H. multicinctus*, 10 mL of each humic acid concentration was poured into small Petri dishes to which 1 mL suspension containing 100 ± 5 number of *H. multicinctus* was added. One mL nematode suspension in 10 mL sterile distilled water served as control. The number of dead nematodes were counted after 24, 48 and 72 h exposure and expressed as percent mortality. The nematodes were considered dead if they did not move on probing with a fine needle. The experiment was conducted twice in a completely randomized design with five replications per treatment.

Pot experiment

Humic acid at 1, 2, 5 and 10% were evaluated as a soil treatment for their efficacy in suppressing *H. multicinctus* infestation on banana under glasshouse conditions during July to November 2013. Untreated banana plants were maintained as standard check. Five sets of 20 kg steam-sterilized sandy loam soil (87% sand, 8% silt, and 5% clay; pH 7.0) were taken in trays. The 2-L humic acid 1%, 2%, 5% and 10% concentrations were prepared and these suspensions were uniformly mixed with 20 kg soil. The soils were then filled in 15-cm diameter pots at 5 kg in each pot and 4 pots for each treatment were maintained. For untreated control, 2 L tap water was mixed in the soil. Immediately after imposing the treatments, tissue culture banana plantlets cv. Grand Naine (AAA) obtained from M/s. Spic Agro Biotech, Coimbatore, India, were planted in pots and maintained in the glasshouse. *Helicotylenchus multicinctus* was introduced around the banana roots at a rate of 5,000 per pot immediately after planting. Each treatment was replicated four times, and the treatments were arranged in a randomized complete block design. The plants were maintained at 28 ± 4 °C in a glasshouse and irrigated once in a day. Plants were fertilized with 20-20-20 (N-P-K) fertilizer at 0.1% concentration at twenty days intervals. No pest or disease was recorded during the study.

The plants were carefully uprooted at 90 days after planting. The number of leaves was counted and expressed as number $plant^{-1}$. The shoot length was measured from the base of the pseudostem to the tip of the last opened leaf and expressed in cm. The root length was recorded from the base of the pseudostem to the tip of the root and expressed in cm. The plant shoot portion was cut at the base of the pseudostem and weighted as shoot weight and the remaining sucker with roots weighted as root weight and expressed in g. Five functional primary roots at least 10 cm long were selected at random from each replication. The lengths of the five selected functional roots were all reduced to 10 cm and the roots sliced lengthwise. The percentage of root lesions was assessed in one half of each of the five roots. The maximum root lesion index given per root half was 20, giving a maximum root lesion index of 100 (percent) for all five together (Das *et al*., 2014). Soil samples of 200 g were taken and processed by Cobb's sieving and modified Baermann funnel method (Southey, 1986). One g root sample was collected randomly from each replication and nematode extraction was done by macerating three times for 10 s (separated by 5 s intervals). The suspension was poured through 300 and 40 µm sieves and rinsed with tap water. The nematodes were collected from 40 µm sieve with 200 mL distilled water and the nematodes were counted using a stereomicroscope. Soil nematode population observed per 200 g soil was converted for 5 kg soil by multiplying nematode population per 200 g by 25. Nematode population in roots was calculated by multiplying the number of nematodes in 1-g root by the total weight of the roots. The sum of the nematode calculated per root system and nematode population estimated in the soil per pot was considered as the final population (Pf). The reproduction factor (Rf) was calculated by dividing the final total nematode (Pf) by the initial inoculum number (Pi) (Seenivasan *et al*., 2012). The experiment was repeated during January to June 2014 to confirm the bio-efficacy of humic acid.

Biochemical studies

To study the biochemical changes caused by humic acid in banana roots, a pot experiment was carried in a randomized complete block design at the Department of Nematology, Tamil Nadu Agricultural University (TNAU), Coimbatore, India with the following treatments replicated five times in June 2014:

(1) T1 – Humic acid at 2% alone;
(2) T2 – Nematode alone;
(3) T3 – Humic acid + nematode;
(4) Untreated control.

The tissue culture banana plantlets cv. Grand Naine (AAA) were planted in 15-cm diameter pots filled with 5 kg of a steam-sterilized pot mixture (red soil:sand:farmyard manure, 2:1:1, v/v) and eight pots or plants were maintained for each replication. Five days after planting, the humic acid 2% drenching was done. On the same day mixed life stages of *H. multicinctus* were inoculated in the root zone at a density of 5,000 pot^{-1}. One plant from each replicate was uprooted on the date of inoculation followed by 24, 48, 72, 96, 120, 144 and 216 h after inoculation. The roots were thoroughly washed under running tap water to remove the adhering soil. From this, five sets of one g fresh roots were surface

sterilized in 0.1% mercuric chloride solution and then repeatedly washed with double distilled water and used for biochemical analysis such as peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase enzyme activities and phenol content. The experiment was repeated once in July 2014.

Assay of peroxidase (PO)

Peroxidase activity was analyzed spectrophotometrically according to the procedure given by Hammerschmidt *et al.* (1982). The enzyme extracts from roots of banana plants were prepared by homogenizing 1 g sample in 2 mL of 0.1 M sodium phosphate buffer (pH 6.5) at 4 °C. The homogenate was centrifuged at 10,000 rpm at 4 °C for 10 min. The supernatant obtained served as an enzyme source. The reaction mixture consisted of 1.5 mL of 0.05 M pyrogallol and 0.1 mL enzyme extract and 0.5 mL of 1% H_2O_2. The change in absorbance was recorded at 420 nm at 30 s intervals for 3 min. The enzyme activity was expressed as change in absorbance min^{-1} g^{-1} fresh weight (Hammerschmidt *et al.*, 1982).

Assay of polyphenoloxidase (PPO)

One gram of sample was homogenized in 2 mL of 0.1 M sodium phosphate buffer (pH 6.5) at 4 °C. The homogenate was centrifuged at 20,000 rpm for 15 min at 4 °C. The supernatant served as enzyme source and PPO activity was determined as per the procedure given by Mayer *et al.* (1965). The reaction mixture consisted of 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µL of the enzyme extract. To start the reaction, 200 µL of 0.01M catechol was added and the activity was expressed as change in absorbance at 490 nm min^{-1} g^{-1} fresh tissue.

Assay of phenylalanine ammonia-lyase (PAL)

One gram of root sample was homogenized in 3 mL ice cold 0.1 M sodium borate buffer, pH 7.0, containing 1.4 mM of 2-mercaptoethanol and 50 mg of insoluble polyvinylpyrrolidone (PVP). The resulting extract was filtered through cheese cloth and the filtrate was centrifuged at 20,000 rpm for 15 min at 4 °C and the supernatant was used as the enzyme source. The PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. Sample containing 0.4 mL of the enzyme extract was incubated with 0.5 mL of 0.1 M borate buffer at pH 8.8 and 0.5 mL of 12 mM L-phenylalanine in the same buffer for 30 min at 30 °C. The amount of trans-cinnamic acid synthesized was calculated using its extinction coefficient of 9,630 M^{-1} cm^{-1} (Dickerson *et al.*, 1984). Enzyme activity was expressed in fresh weight basis as nmol trans-cinnamic acid min^{-1} g^{-1} fresh tissue.

Estimation of total phenols

Phenol content was estimated as per the procedure given by Zieslin and Ben-Zaken (1993). One gram root tissue was homogenized in 10 mL of 80% methanol with pestle and mortar and agitated for 15 min at 70 °C. One mL of the methanolic extract was added to 5 mL distilled water and 250 µL Folin-Ciocalteu reagent (1N), and the solution was kept at 25 °C. After 3 min, 1 mL of a saturated solution of sodium carbonate and 1 mL distilled water were added and the reaction mixture was incubated for 1 h at 25 °C. The absorption of the blue color was measured using a UV-visible spectrophotometer (Model Varian Cary 50, Victoria, Australia) at 726 nm. The content of the total soluble phenols was calculated according to a standard curve obtained from the Folin-Ciocalteu reagent with a phenol solution (C_6H_6O) and expressed as catechol equivalents g^{-1} tissue.

Statistical analysis

Data from the identical experiments were pooled when statistically appropriate according to the Student's *t*-test. The data were analyzed using analysis of variance and treatment means were compared by the Duncan Multiple Range test (Panse and Sukhatme, 1989). Percentage data were arcsine transformed [y = arcsin(x)] before analysis. The software used for analysis was SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results and discussion

***In vitro* studies**

Humic acid had a significant effect on mortality of *Helicotylenchus multicinctus* when compared to water control (Figure 1). The nematode mortality rate was more at higher con-

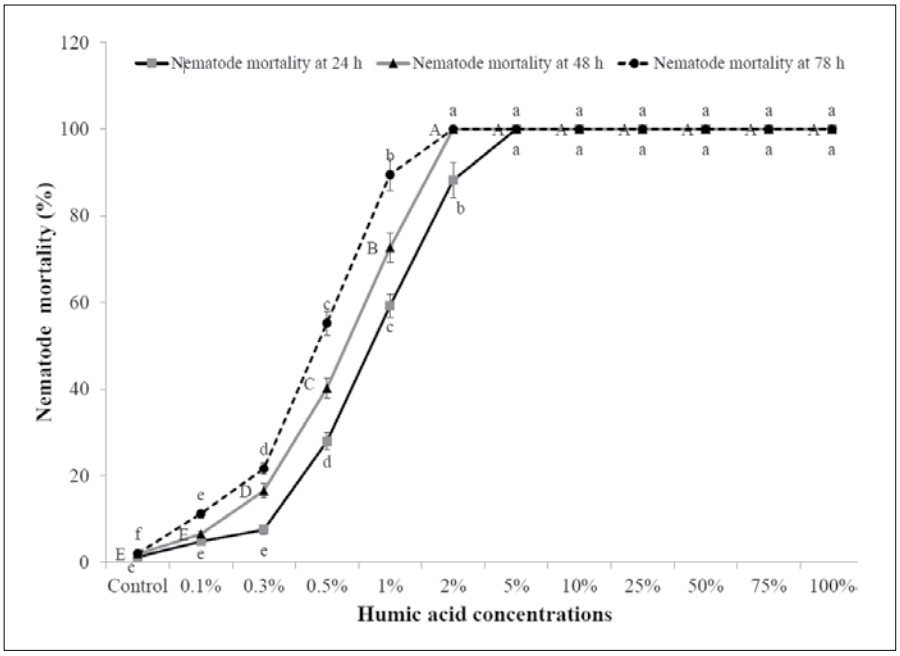

Figure 1. Mean mortality of *Helicotylenchus multicinctus* after exposure to different concentrations of humic acid. Bars on the lines represent ± SE of the means (n = 5). Bars with the same letter do not differ significantly according to Duncan's multiple range test (P < 0.05).

centrations than at lower concentrations. The mortality rate reaches 100% when exposed to humic acid at concentrations > 5% after 24 h exposure. Humic acid 2% (T1) caused 88.2% mortality 24 h after exposure and reached 100% mortality after 48 h exposure. Nematode mortality was 4.8–89.5% in 0.1–1.0% humic acid concentrations at 24–72 h exposure. In general, the percentage of mortality increased with increasing concentration of humic acid and also with increasing exposure time. The suppressive effect of some organic acids on nematode populations has been well documented in several patho-systems (Chitwood, 2002). This is the first report on the use of humic acid for the control of *H. multicinctus* infesting banana. The present results obtained were in accordance with Nandi *et al.* (2000) and Zaki *et al.* (2004). They reported that nematicidal potential of various organic acids such as myristic acid, palmitic acid, oleic acids, fulvic acid, acetic acid, N-bulyric acid, formic acid, lactic acid and propionic acids against various nematodes such as *Aphelenchus avenae*, *Aphelenchoides goodey*, *Helicotylenchus pseudorobustus*, *Meloidogyne hapla* and *Xiphinema americanum*. Dias and Ferraz (2001) also recorded 90% mortality of *Heterodera glycines* due to humic acid followed by the application and decomposition of poultry manure. Similar lethal effect of humic acid on egg hatching and juvenile mortality of *M. incognita* was observed by various workers (Saravanapriya and Subramanian, 2007; Jothi *et al*., 2009; Dias *et al*., 1999). It is suggested that the nematicidal property of humic acid could be attributed to certain active principles present in humic acid. The humic acid mainly contains carboxyl, phenolic, alcoholic hydroxyl and carbonyl groups (Hansen and Schnitzer, 1969). Since the greatest nematicidal activity reported in the chemical compounds containing carboxyl and phenolic group (Chitwood, 2002), the presence of carboxyl and phenolic functional groups in humic acid might be the possible reason for the lethal effect on *H. multicinctus*.

Pot studies

The final population of nematodes in soil and root was significantly less in the humic acid treated plants than in the control ones (Table 1). Humic acid treatments at all concentrations (1, 2, 5 and 10%) reduced *H. multicinctus* in the soil by 53.6–56.6% and in the roots by 39.1–44.5% compared to the untreated plants. Multiplication of *H. multicinctus* was significantly higher in control plants with 30.3 reproduction factor. It was significantly reduced (42.9–47.1) by the application of 1, 2, 5 or 10% humic acid compared to the untreated plants. However, the multiplication rate was not significantly different between the various treatments. The root lesion index was less (16.0–17.3%) in the humic acid treated plants versus more (30.3%) in the control plants. All the tested concentrations were equally effective in reduction of soil, root population, multiplication rate and root damage. The results of our *in vitro* tests confirmed that humic acid had a direct antagonism against *H. multicinctus*. Hence, the mixed stages of *H. multicinctus* in soil might be killed when directly exposed to humic acid and resulted in reduction of their infestation and multiplication on banana roots. These results are supported with those obtained by Saravanapriya and Subramanian (2007). They showed that soil amended with humic acid decreased significantly the number of galls, number of egg masses per plant and final soil population of *M. incognita* in tomato. In this study, humic acid soil drenching at 1, 2, 5 and 10% concentrations were found to be equally effective to suppress the *H. multicinctus.* The findings of Jothi *et al.* (2009) support our results who reported that 0.4–1.0% concentration of humic acid is sufficient to cause 93–100% mortality of *M. incognita* juveniles under *in vitro* conditions.

Growth of banana plantlets in terms of number of leaves, pseudostem length, root length, pseudostem girth, fresh pseudostem weight and fresh root weight was affected by *H. multicinctus* infestation in the absence of humic acid treatments (Table 2). Conversely, growth of banana plantlets infested by *H. multicinctus* grown in soil treated with humic acid was significantly improved irrespective of different concentrations tested, when compared with untreated control. The decrease in nematode population and root infestation in humic acid treated plants may have contributed to this increased growth. The humic acid treatments at 1, 2, 5 or 10% concentration increased the number of leaves by 15.2–16.4%, pseudostem height by 19.3–22.1%, pseudostem girth by 11.9–13.2%, pseudostem weight by 22.8–23.9%, root length by 20.0–22.8% and root weight by 17.2–20.4% against the control. However, number of leaves, pseudostem length, root length, pseudostem girth, pseudostem weight and root weight were not significantly ($P > 0.05$) different between the concentrations of humic acid applied in this experiment. Similar improved growth and yield due to humic acid was reported by several investigators on various crops such as tomato (Saravanapriya and Subramanian, 2007), wheat (Ulukan, 2008), cucumber (El-Nemr *et al*., 2012), pomegranate (Khattab *et al*., 2012) and peas (Khan *et al*., 2013). Humic acid has also been reported to enhance mineral nutrient uptake by plants, by increasing the permeability of membranes of the root cells (Valdrighi *et al*., 1996). The positive influence of humic acid on banana growth could also be mainly due to hormone-like activities of the humic acids through their involvement in cell respiration, photosynthesis, and various enzymatic reactions (Atiyeh *et al*., 2002).

Biochemical studies

Peroxidase (PO)

The peroxidase activity was triggered in banana roots due *to H. multicinctus*, humic acid and *H. multicinctus* + hu-

Table 1. Effect of humic acid on *Helicotylenchus multicinctus* infestation (mixed population of J2, J3, J4 and adults) in banana plantlets *Musa acuminata* (AAA) cv. Grand Naine. Values are means ± standard errors ($n = 6$) (Rf: Reproduction factor).

Treatments	Nematode population in soil (100 cm³)	Nematode population in root (1 g)	Rf	Root lesion index
Humic acid 1%	37.9 ± 1.7 b[y]	175.9 ± 7.3 b	1.3 ± 0.3 b	17.3 ± 0.7 b
Humic acid 2%	36.8 ± 2.1 b	169.3 ± 8.1 b	1.3 ± 0.3 b	17.3 ± 1.1 b
Humic acid 5%	35.7 ± 1.7 b	160.9 ± 7.6 b	1.2 ± 0.1 b	16.0 ± 0.7 b
Humic acid 10%	35.4 ± 2.3 b	160.3 ± 8.3 b	1.2 ± 0.1 b	16.0 ± 0.7 b
Control	81.7 ± 3.7 a	289.0 ± 11.7 a	2.1 ± 0.3 a	30.3 ± 1.7 a

[y] Means followed by the same letter in a column are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 2. Effect of humic acid on growth parameters such as number of leaves, pseudostem length, root length, pseudostem girth, pseudostem weight and root of banana plantlets (*Musa acuminata* (AAA) cv. Grand Naine) infected with *Helicotylenchus multicinctus*. Values are means ± standard errors (n = 6).

Treatments	Number of leaves	Pseudostem length (cm)	Root length (cm)	Pseudostem girth (cm)	Pseudostem weight (g)	Root weight (g)
Humic acid 1%	7.2 ± 0.3 a[y]	53.1 ± 2.1 a	40.5 ± 1.6 a	6.7 ± 0.5 a	125.0 ± 6.7 a	27.2 ± 1.8 a
Humic acid 2%	7.2 ± 0.7 a	53.8 ± 1.9 a	41.1 ± 1.9 a	6.7 ± 0.8 a	125.3 ± 7.3 a	27.6 ± 1.4 a
Humic acid 5%	7.3 ± 0.3 a	54.9 ± 2.6 a	41.4 ± 1.4 a	6.8 ± 0.6 a	126.6 ± 9.3 a	28.1 ± 1.7 a
Humic acid 10%	7.3 ± 0.3 a	55.0 ± 2.3 a	42.0 ± 2.3 a	6.8 ± 0.7 a	126.9 ± 8.7 a	28.3 ± 1.9 a
Control	6.1 ± 0.3 b	42.8 ± 2.2 b	32.4 ± 1.7 a	5.9 ± 0.4 b	96.5 ± 6.1 b	22.5 ± 1.9 b

[y] Means followed by the same letter in a column are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

mic acid inoculation (Figure 2). Induction of PO was higher in humic acid + nematode treated plants challenge inoculated with *H. multicinctus* (T3). The peroxidase activity ranged from 0.93 to 3.30 A_{420} min^{-1} g^{-1} fresh tissue in the T3 plants. Individual treatment with humic acid alone (T1) and nematode alone (T2) showed moderate induction of PO activity. PO activity in control plants recorded lower activity of 0.91–0.94 changes in A_{420} min^{-1} g^{-1} fresh tissue. The activity of PO was noticed up to 120 h and thereafter started to decline. PO is more important as it is the first enzyme in the phenylpropanoid pathway, which leads to production of phytoalexin and phenolic substances, leading the formation of lignin (Bruce and West, 1989). PO activity in roots is also important in the reinforcement of cell walls at the border of infection in resistant plants and that are considered as important components of active defense response of nematode invaded tissue (Zacheo *et al*., 1995).

Polyphenoloxidase (PPO)

The humic acid + nematode treatment (T3) expressed the maximum polyphenol oxidase activity (1.96–2.73 A_{490} min^{-1} g^{-1}). The minimum of 1.63–1.67 A_{490} min^{-1} g^{-1} was registered in the control plants. Polyphenol activity was the highest in T3 (21.3–30.5% higher than the control). The induction of PPO was noticed up to 120 h and thereafter started to decline. Individual treatments with humic acid alone and nematode alone showed moderate induction of PPO activity (Figure 3). PPO oxidizes the phenols to highly toxic quinones and hence is considered to play an important role in disease resistance, particularly those affecting the tissues (Abbattista and Matta, 1975).

Phenylalanine ammonia-lyase (PAL)

The induction of PAL was noticed in *H. multicinctus*, humic acid and *H. multicinctus* + humic acid inoculated plants up to 120 h and thereafter started to decline (Figure 4). Induction of PAL was higher (7.40–20.99 nm transcinnamic acid min^{1} g^{-1}) in the humic acid treated plants challenge inoculated with *H. multicinctus*. The phenylalanine ammonia-lyase activity was low (5.60–5.92 nm transcinnamic acid min^{-1} g^{-1}) in the control plants. T1 and T2 plants also expressed induction of PAL activity. However, the percent increase over control was the highest in T3 plants (31.3–71.7%). PAL is another important enzyme involved in the synthesis of phenolics, phytoalexin and lignin. The increased activity of PAL was triggered in the infected clones to reinforce the integrity of the cell wall by encoding lignin biosynthesis. Lignin and wall bound phenolics are synthesized in the phenylpropanoid pathway. PAL is the first enzyme in the phenylpropanoid pathway and thus PAL is involved in the defense mechanism of the plant (Kathiresan and Mehta, 2005).

Total phenols

The total phenol content was significantly higher (19.84 µg glucose min^{-1} g^{-1} fresh tissue) in the humic acid treated banana plants inoculated with *H. multicinctus* than

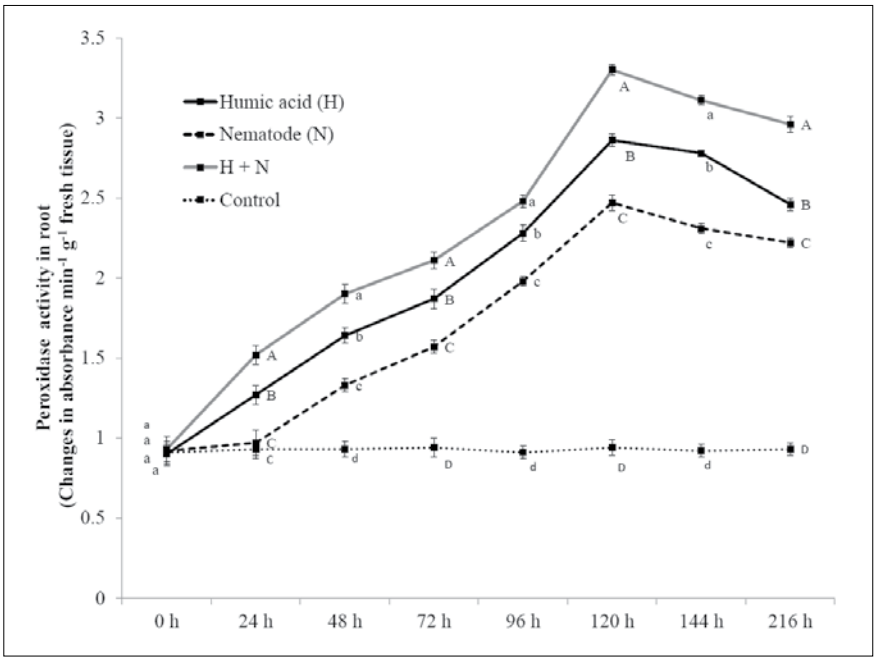

Figure 2. Changes over time in peroxidase (PO) activity by humic acid in banana (*Musa acuminata* (AAA) cv. Grand Naine) roots challenge inoculated with *Helicotylenchus multicinctus.*
Bars on the lines represent ± SE of the means (n = 5). In each column, bars followed by a common letter do not differ significantly ($P < 0.05$).

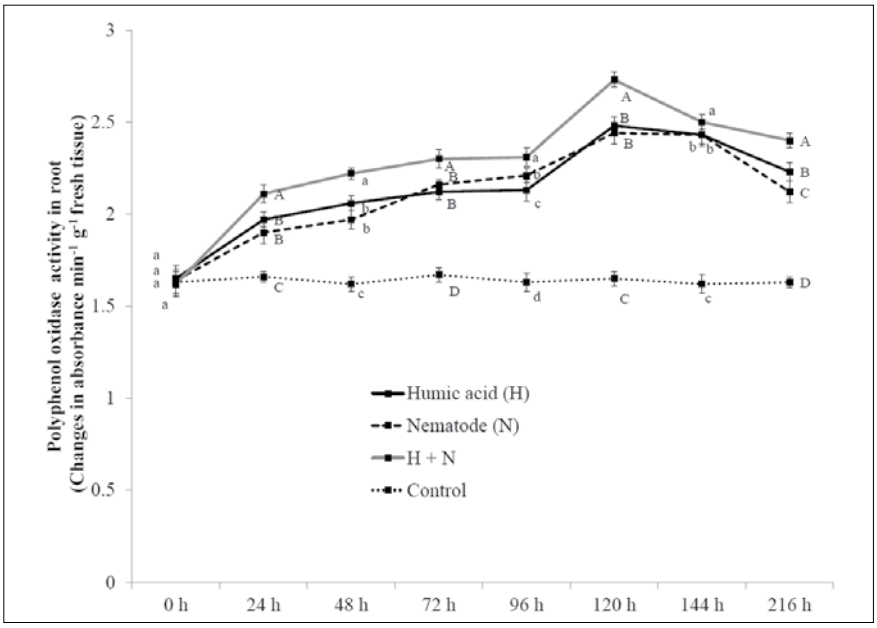

Figure 3. Changes over time in polyphenol oxidase (PPO) activity by humic acid in banana (*Musa acuminata* (AAA) cv. Grand Naine) roots challenge inoculated with *Helicotylenchus multicinctus*. Bars on the lines represent ± SE of the means (n = 5). In each column, bars followed by a common letter do not differ significantly (P < 0.05).

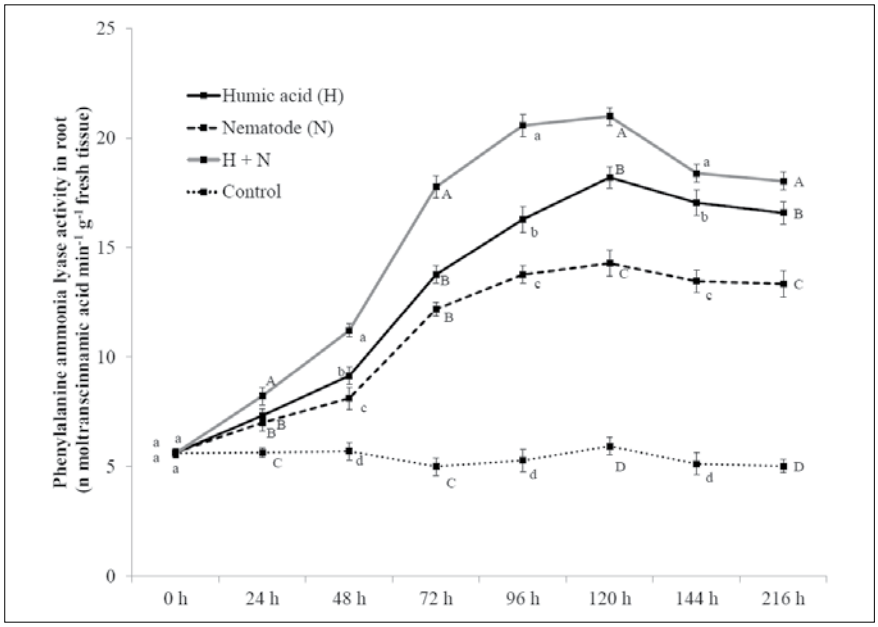

Figure 4. Changes over time in phenylalanine ammonia-lyase (PAL) activity by humic acid in banana (*Musa acuminata* (AAA) cv. Grand Naine) roots challenge inoculated with *Helicotylenchus multicinctus*. Bars on the lines represent ± SE of the means (n = 5). In each column, bars followed by a common letter do not differ significantly (P < 0.05).

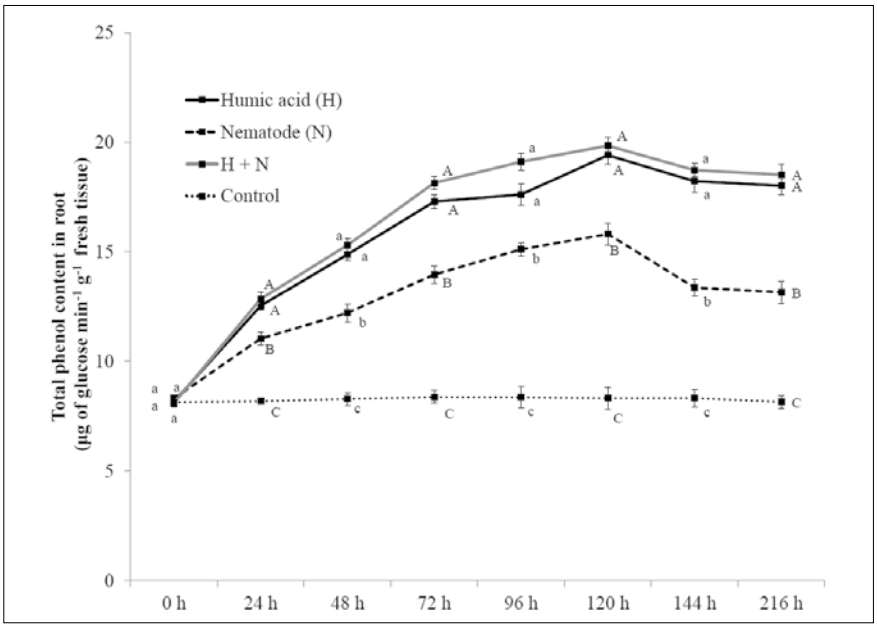

Figure 5. Change in total phenol content by humic acid in banana (*Musa acuminata* (AAA) cv. Grand Naine) roots challenge inoculated with *Helicotylenchus multicinctus*. Bars on the lines represent ± SE of the means (n = 5). In each column, bars followed by a common letter do not differ significantly (P < 0.05)

in the treatment with nematode alone or in the untreated control (Figure 5). The lowest phenol contents of 8.13–8.37 µg glucose min^{-1} g^{-1} fresh tissue were recorded in the control plants. Percent increase in total phenol content over the control was maximum in T3 (36.4–58.1%) and minimum in T2 (26.0–47.4%). Vidhyasekaran (1988) described the occurrence of many kinds of phenolics in plants. Among them, total phenols play a unique role in response to pathogen and nematode invasion. The accumulation of phenol may be due to the excess production of hydrogen peroxide by increased respiration or due to the activation of hexose monophosphate shunt pathway, acetate pathway and release of bound phenols by hydrolytic enzymes. The results of the present study revealed a significant increase in phenol content in the humic acid treated plants challenge inoculated with *H. multicinctus*. Enhanced root phenol content has been associated with banana varieties or hybrids resistant to *H. multicinctus* (Das *et al*., 2014).

Plants are endowed with defense genes which are quiescent in healthy plants. When these genes are activated by various factors they induce systemic resistance against nematodes. Induced systemic resistance activates multiple defense mechanisms that include increased activity of pathogenesis related proteins like PO, PPO and PAL; and phenol content (Das *et al*., 2014; Seenivasan *et al*., 2012; Seenivasan, 2011). The biochemical analysis estimated in the current investigation showed that the humic acid treated plants challenge inoculated with *H. multicinctus* possessed higher PO, PPO, PAL activity and phenol content than the humic acid alone (T1) or nematode alone (T2), indicating some induced systemic resistance mechanism due to humic acid. Hence the reason for the improved nematode control in humic acid treated banana plantlets may be due not only to a blockage of early root penetration of *H. multicinctus* through direct nematicidal action, and also to deterring the development and reproduction potential of penetrated *H. multicinctus* in the banana roots through some systemic resistance induction.

Conclusion

Eco-friendly pest and disease management involving organic materials is increasingly practiced in the actual scenario of resilient agriculture. Soil drenching with 1–2% humic acid seems to be sufficient to get a significant control of nematodes as well as to improve the growth of the banana plant. There is a growing interest in the use of humic acid as organic fertilizer since it can be easily applied through drip irrigation as fertigation in most banana plantations. The banana planters are now switching from a system of flood irrigation to a drip irrigation one due to water and labor scarcity. Hence findings such as the application of humic acid at 1–2% could be deployed for the protection against the *Helicotylenchus multicinctus* menace in banana. Fertigation with "pestigation" technology shall now be tested under field conditions.

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