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IMPACT OF HUMIC AND FULVIC ACIDS ON CONTROLLING *Meledogyne incognita* INFESTING TOMATO AND COW PEA PLANTS

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ABSTRACT

Humic and fulvic acids were extracted from compost and biogas manure by alkaline (either NaOH 0.5 N or KOH 1 N), and tested against the root – knot nematode, *Meledogyne incognita*, *in vitro* and *in vivo* on both tomato and cow pea and compared with the nematicide, vydate. *In vitro* vydate at double application dose was the best treatment for inhibiting hatch (47.37%, 45.7% inhibition) with humic and fulvic acid respectively, and was significantly more effective than all others treatments, Humic acid extracted from biogas by NaOH (1 ml L^{-1}) was the least effective treatment for inhibating hatch, but humic extracted from compost by KOH (2 ml L^{-1}) was significantly better than the other treatments in reducing the number of surviving juveniles. Humic acid achieved the highest percentage of nematode inhibition (26.09%) in compared with fulvic acid extracted from compost by NaOH in which it was low effective for inhibiting hatch. On the other hand fulvic acid extracted from biogas by KOH was the best treatment in reducing the number of surviving juveniles and achieved up to 45.1 % inhibition and was significantly more effective than all others treatments. *In vivo,* fulvic acid (2 ml L^{-1}) significantly reduced the numbers of galls, final population, population build- up (Pf/Pi) and nematode reduction percentage. About 1 ml of fulvic acid indicated all best treatment, and the application of both humic acid was nearly effective as Vydate (double treatment). The double application of humic acid gave the best plant growth (fresh and dry weights) in cow pea and tomato plants. All treatments reduced nitrogen content in plants (tomato and cow pea plants), but fulvic acid once or twice dose increased phosphorus content in tomato plant more than vydate. Potassium content in tomato plants was increased in vydate treatment than in the other treatments.

Keywords: Humic acids, fulvic acids, compost, *Meledogyne incognita,* tomato, cow pea.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) cause worldwide a major economic damage in agricultural production. The main options for controling of phytoparasitic nematodes include chemical nematicides, crop rotation and resistant cultivars when available. The broad host spectrum of *Meloidogyne* species makes all crop rotation difficult. Fumigant nematicides, although they were effective, but they have negative side effects that have led to their ban or restricted use. Resistance breaking populations of *Meloidogyne* are challenging the use of resistant cultivars (Castagnone-Sereno, 2002a and b and Robertson *et al*., 2006).

Humic substances (HS) are natural organic compounds comprising from 50 to 90% of the organic matter of peat, lignites, sapropels, composts, as well as of the non-living organic matter of soil and water ecosystems (Clapp *et al*., 1993). The functional groups of the HS, which determine the physical and chemical characteristics, vary and depend on the origin and age of the material (Gaffney *et al*., 1996). Such substances comprise three basic components: humins, humic acids (HA), and fulvic acids (FA). These components are traditionally defined according to their solubility. Humins are the fraction, which is insoluble at all pHs, humic acids are insoluble at pHs below pH 2.0, and fulvic acids are soluble at all pHs. Humic substances are thought to consist of a skeleton of alkyl or aromatic units cross-linked mainly by oxygen and nitrogen groups, with the major functional groups being carboxylic acid, phenolic and alcoholic hydroxyls, ketone, and quinone groups (Schulten *et al*., 1991). This structure allows HS to bind both hydrophobic and hydrophilic materials, and thus they play an important role in nematode resistance. The objective of study was to determine the effect of HS on the reproduction of *Meloidogyne incognita,* the major parasite in vegetable fields in Egypt.

The present work is carried out to study the utilization of fulvic and humic acid after extraction from the different types of compost and their role in controling root-knot nematodes in infected soil of tomato and cow pea, which caused by *Meledogyne incognita,* using fulvic and humic acid with two levels of addition with and/or without vydate as chemical treatment in comparison with control (non inoculated soil).

MATERIALS AND METHODS

Extraction and purification of humic and fulvic acids:

Extraction of humic and fulvic acids was run according to the method as described by Sanchez *et al.* (2002). The compost samples were treated with either 0.5 N NaOH or 1.0 N KOH (Bidegain *et al.,* 2000). The obtained materials still contain impurities, which purified as described by Chen *et al.* (1978). While the purification of fulvic acid was completed according to as described the method by Kononva (1966).

Effect of humic substances on nematode *in vitro*:

Galled tomato roots from the field were transferred into polyethylene bags to laboratory (Nematology Research Center, Faculty of Agriculture, Cairo University). After identification by the perineal pattern of mature females (Taylor and Sasser, 1978), a single egg mass was used to inoculate sunflower plants cv. Miak grown in 20 cm diameter pots. The pots were filled with sterilized sandy loam soil (1:1 v/v). Tow months after inoculation, plants were removed from the pots and examined for nematode infection. A pure culture of *Meloidogyne incognita* was maintained on sunflower, using repeated inoculations to obtain a sufficient quantity of inoculum for the experiments. To test the effect of humic and fulvic acids on hatch of *M. incogntia*, ten egg masses of uniform size were placed on a small pieces of foam (2 × 2 × 1 cm) and were immersed in 15 ml of each treatment concentration (0, 2, 4 ml l^{-1} water) dissolved in sterile distilled water. Each treatment was contained in a 9 cm diameter Petri dish and all treatments were replicated three times. The test was conducted at room temperature (20ºC). Hatched second stage juveniles (J2) were withdrawn and counted at intervals of 24, 48 and 96 h respectivly. Humic or fulvic acid solutions were added after each withdrawal of J2. The percentage hatch inhibition compared with controls was determined. To test the effect it's on the survival of J2, 1800 ± 100 freshly hatched J2 (1- day – old) were placed in 15 ml of the same products / concentrations that were used in the hatching test. Each chemical concentration was replicated three times. The test was conducted at room temperature (20ºC). Total (live and dead) juveniles were counted at the same tested intervals as the hatching test. Percentage mortality was estimated by counting the number of dead nematode as those showing no movement and having a stick – like shape; these were transferred to distilled water to confirm that they were dead and not moribund.

Effecte of humic substances on Nematode *in vivo*:

One month old tomato seedlings cv. *Castle rock* and Cow pea were transplanted into 15 cm diameter. Pots filled with steam sterilized soil sandy loam (coarse sand 30.2 %, fine sand 55.6 %, silt 8.2 %, and clay 6 %). The seedling were inoculated with 2000 J2 of *M. incognita* / pot by pipetting the inocula in three holes around the root system. One week after inoculation, the following materials were added to soil: humic acid, fulvic acid (1g/L) and Vydate (w/v) (10% active substance as a liquid formation) as a control. the chemical were added as soil drenches (200 ml/ pot), either in a single treatment at the rate of 10 ml $^{-1}$ water or in two treatment at the rate of 5 ml^{-1} water at intervals of 2 weeks. Each treatment was replicated five times, including untreated inoculated and non inoculated pots that served as controls. All treatments were arranged in fully randomized design on a clean bench in the glasshouse at 32 ± 5ºC, and received similar horticultural treatments. Two month after inoculation, plants were removed from pots and data plant growth (total fresh weight and total dry weight) were recorded. Nematodes were extracted from the soil of each pot by decanting and sieving according to Baermann- pan technique (Hooper *et al.,* 2005). The nematodes from each pot were counted in a Hawksley counting slide, under a binocular microscope. A subsample (3 g) of roots from each plant was stained with acid fushsin / lactophenol (Hooper *et al.,* 2005) and the numbers of galls and stages embedded in roots (developmental stages + egg masses) per root were counted. The final population (embedded stages + nematodes in soil), rate of build – up population final (pf)/ population initial (pi), and percentage of nematode reduction were then calculated.

Plant analysis:

Total nitrogen was determined in soils, plants, agricultural wastes and in compost materials using Kjeldahl digestion method reported by Jackson (1973). Total phosphorus content using ascorbic acid as a reluctant (Murphy and Riley, 1962). Digest solutions of soil, compost and plant samples were used for determination of total potassium content by flame photometrically (Chapman and Pratt, 1961).

Elemental analysis (C, H, N, S and O_2 %) of humic and fulvic acid:

Elemental analysis for carbon, hydrogen, nitrogen and sulphur contents of the purified humic and fulvic acids was performed by gas chromatography on a Hewlett-Packard 185 (C, H, N, S automatic) microanalyser (Vario Elmentor/C, H, N, S Germany) 2004 (Micro Analytical Center-Faculty of Science, Cairo University). Oxygen was calculated by deference (summation of all constituents from 100) (Goh and Stevenson, 1971).

Determination of total acidity of humic and fulvic acids:

Total acidity of humic and fulvic acids was determined following the method described by Dragunova (1958).

Determination of COOH group:

Carboxyl groups of humic and fulvic acids were determined by calcium acetate $(CH_3COO)_2$ Ca method mentioned by Schnitzer and Gupta (1965).

Phenolic hydroxyl groups:

Phenolic OH groups were determined by subtracting COOH group's content from total acidity according to Kononova (1966).

Statistical analysis:

The statistical analysis for the obtained data were computed using analysis of variance procedure described by Sendecor and Cochran (1980), the significant mean differences between treatment means were separated by Duncan׳s Multiple Range Test (Duncan, 1955).

RESULTS

***In vitro* tests:**

The data obtained in Tables 1 & 2 indicated the concentration of Vydate (2 ml) was the best treatment for inhibiting hatch (47.37 and 45.7 inhibition) than humic and fulvic, respectively. Also it was more significantly more effective than all other treatments, except for Humic acid extracted by NaOH (0.5 N) at the lower concentration. Humic acid extracted by KOH (1.0 N) from biogas manure (HA Bio. m.) (1ml L^{-1}) was less effective as well as fulvic acid extracted by NaOH (0.5N) from compost (FA comp.) (1, 2 ml L^{-1}). The vydate at 2 ml L^{-1}gave the highest percent mortality of the hatched juveniles (38.3) and it was surpassed all fulvic treatments (Table2) in comparison with humic acid (HA Bio. m.), which it was higher than vydate at 2 ml and other treatments achieved (35.39 %) percent mortality (Table 1), followed by HA Bio m at low dose (35.21) mortality. The least nematode mortality was in the low concentration of HA comp at 1 ml and FA comp 2 ml in extracted by NaOH.

Data in Tables (1, 2) indicated that the concentration of (2 ml) of FA Bio.m (KOH) was significantly better than other products in reducing the number of surviving J2 and achieved the highest percentage of nematode inhibition (45.1%), followed by the two concentrations of HA comp. (KOH), and vydate (Table 2), which were not significantly different from each another. In contrasts, 1 ml concentration of FA comp (KOH) resulted in an adverse effect, giving the lowest percentage of nematode inhibition, followed by vydate (Table 1), although the differences between both treatments were not significant.

Vydate at a concentration of 2 ml in (Tables 1&2) increased percentage of mortality and FA Bio m, at the same concentration gave 38.5 % mortality. In general, treatment with either humic or fulvic acid was effective at high concentrations against the nematicide vydate in reducing hatching and survival of J2.

Table (1): Effect of humic acids on hatching and survival percentage of nematode (*Meloidogne incognita*) in vitro

Treatment		Dose (ml)	Hatching			Survival		
			Total	Inhibition %	Mortality %	Total	Inhibition %	Mortality %
Na OH 0.5 N	HA Comp.	1.0	2133 [c]	10.90	14.35	912 [bcd]	17.39	13.16
		2.0	1332 [fg]	44.36	14.19	948 [abcd]	14.13	12.66
	HA Bio.m	1.0	2250 [bc]	6.02	16.40	1008 [abc]	8.70	11.27
		2.0	1494 [de]	37.59	16.27	984 [abcd]	10.87	9.76
KOH 1.0 N	HA Comp.	1.0	2160 [bc]	9.77	30.83	1044 [ab]	5.43	14.43
		2.0	1431 [ef]	40.23	30.81	816 [d]	26.09	14.71
	HA Bio.m	1.0	2286 [ab]	4.51	35.21	852 [cd]	22.83	14.67
		2.0	1602 [d]	33.08	35.39	840 [cd]	23.91	22.86
Vydate		1.0	1485 [de]	37.97	27.27	1056 [ab]	4.35	31.82
		2.0	1260 [g]	47.37	29.29	936 [abcd]	15.22	41.03
Nematode only			2394 [a]	0.00	10.90	1104 [a]	0.00	20.60
L.S.D. $_{(0.05)}$			127.010			165.92		

- Each value represents the mean ± S.D (Standard Diviasion) and mean of three replicates.
- Values in the same column with the same letter are not significantly different at ($p \leq 0.05$).
- HA Comp.: Humic acid extracted from compost (plants residual). HA Bio.m: Humic acid extracted from biogas manure.

$$\text{Inhibition \%} = \frac{\text{Total in control} - \text{Total in Treatment}}{\text{Total in control}} \times 100.$$

$$\text{Mortality \%} = \frac{\text{Total dead}}{\text{Total (live + dead)}} \times 100.$$

Table (2): Effect of fulvic acids on hatching and survival percentage of nematode (*Meloidogne incognita*) in vitro

Treatment		Dose (ml)	Hatching			Survival		
			Total	Inhibition%	Mortality%	Total	Inhibition%	Mortality%
Na OH 0.5 N	FA Comp.	1.0	3195 [ab]	3.2	21.2	2100 [bc]	8.5	15.5
		2.0	3195 [ab]	3.2	24.6	1750 [e]	23.8	21.3
	FA Bio.m	1.0	3120 [b]	5.5	24.2	2190 [abc]	4.6	20.9
		2.0	2880 [c]	12.7	26.7	2070 [c]	9.8	27
KOH 1.0 N	FA Comp.	1.0	3150 [ab]	4.5	21.5	2220 [ab]	3.3	13.7
		2.0	3135 [b]	5.0	25.8	1910 [d]	16.8	22.1
	FA Bio.m	1.0	2370 [d]	28.2	22.2	1900 [d]	17.2	31
		2.0	2160 [e]	34.5	24.4	1260 [f]	45.1	38.8
Vydate		1.0	2086 [e]	36.8	28.6	2085 [bc]	9.2	31.5
		2.0	1790 [f]	45.7	38.3	1720 [e]	25.1	42.9
Nematode only			3300 [a]	0.0	0.90	2100 [a]	8.5	6.7
L.S.D. $_{(0.05)}$			142.17			129.09		

- Each value represents the mean ± S.D (Standard Diviasion) and mean of three replicates.
- Values in the same column with the same letter are not significantly different at ($p \leq 0.05$).
- FA Comp.: fulvic acid extracted from compost (plants residual). FA Bio.m: fulvic acid extracted from biogas manure

Reproduction of *M incognata* and plant growth response of tomato and cow pea in vivo:

Humic and fulvic Bio. m. extracted by KOH 1 N were tested in sandy loam soil (Table 3) in pot experiment on tomato plants and cow pea. All treatments significantly ($P \leq 0.05$) reduced the numbers of galls, final population and consequently, the rate of nematode build – up (Pf /Pi), in soil as compared to the untreated control. The vydate at the rate of 1 and 2 ml L^{-1} achieved highest preventing nematode penetration of the root of cow pea than tomato, without significant differences between them. On the other hand fewer galls and stages inside roots were observed on plants grown with vydate than with the other treatment. Fulvic acid (2 ml L^{-1}) was nearly effective as Vydate (double treatment) (Table 2). In cow pea and tomato the double application of fulvic acid resulted in and was significantly higher preventing of nematode root penetration than single application of fulvic acid in reducing the numbers of formed galls at both doses. A single and double application of humic acid with tomato and cow pea were less effective than with fulvic acid in reducing gall numbers, final stages and nematode build-up. Thus, the double application was the best treatment (after the nematicide) for suppressing egg production and diminishing nematode populations.

Table (3): Effect of humic and fulvic acids on (*Meloidogne incognita*) reproduction of tomato and cow pea grown in soil

Treatment	Dose (ml)	Tomato				Cow pea			
		Galls	Final	Pf/pi	Reduction %	Galls	Final	Pf/pi	Reduction %
Humic	1.0	175 b	6539	3.26 d	4.0	125 c	4689	2.43 bc	14.0
Bio. m	2.0	164 b	5247	2.62 c	39.0	108 ab	3641	1.82 b	36.0
Fulvic	1.0	186b	6233	3.12 d	9.0	133 c	5018	2.51 c	8.0
Bio.m	2.0	156 ab	4140	2.06 b	51.6	104 ab	3456	1.73 b	54.0
Vydate	1.0	78 a	2910	1.46 a	57.0	64a	2365	1.18 a	57.0
	2.0	72 a	2432	1.23 a	64.0	60 a	2012	1.01 a	63.0
Nematode		180 b	6821	3.33 d	-	145 d	5433	2.72 d	-
L.S.D. (0.05)		29.593		1.819		14.486		1.966	

- Means followed by the same letter (s) within a column in each block are not different significantly ($p \leq 0.05$) according to duncans multiple range test.
- Pf /pi = population of final nematode pi / population of initial nematode.

The double application of humic acid in soil gave the best plant growth (fresh and dry weights) of tomato plants than cow pea plants (Table 4) followed by the single applications of humic and double application of fulvic acid. The vydate represents significantly lower plant growth results than humic and fulvic acid. Generally, the application of humic and fulvic acid both once or twice gave significantly better plant growth and improved plant healthy than all other treatments.

Table (4): Effect of humic and fulvic acids on tomato and cow pea growth

Treatment	Tomato				Cow pea			
	Fresh	Change %	Dry	Change %	Fresh	Change %	Dry	Change %
Humic (1ml)	21.1 [ab]	+ 128.3	11.6 [b]	+ 110.9	24.8 [a]	+ 54.4	18.5 [b]	+ 110.2
Humic (2ml)	23.7 [a]	+ 155.4	14.1 [a]	+ 156.4	27.5 [a]	+ 73.1	22.3 [a]	+ 152.3
Fulvic (1 ml)	15.4 [c]	+ 63.0	9.0 [c]	+ 63.6	17.7 [c]	+ 10.6	14.2 [cd]	+ 61.4
Fulvic (2 ml)	17.1b [c]	+ 85.9	12.0 [b]	+ 118.2	21.1 [b]	+ 31.9	16.5 [bc]	+ 92.0
Vydate (1 ml)	13.6 [cd]	+ 44.6	8.6 [c]	+ 56.4	17.0 [c]	+ 6.3	12.9 [de]	+ 45.5
Vydate (2 ml)	14.9 [c]	+ 6.9	7.8 [cd]	+ 41.8	18.0 [c]	+ 13.1	13.6 [d]	+ 54.5
Healthy	11.9 [cd]	+ 28.3	7.1 [d]	+ 29.1	17.2 [c]	+ 7.5	10.8 [ef]	+ 22.7
Nematode	9.03 [d]	-	5.5 [e]	-	16·0 [c]	-	8.9 [f]	-
LSD	5.197		1.341		2.833		2.518	

- Means followed by the same letter (s) within a column in each block are not significantly different ($p \leq 0.05$) according to duncans multiple range test.

All treatments of humic and fulvic acids reduced plant content of nitrogen in tomato and cow pea plants (Table 5). On the other hand fulvic acid once or twice increased the content of phosphorus in tomato plant more than vydate. Potassium content in tomato was increased in vydate treatment than all treatment. In contrast all treatments increased k content in cow pea but the single dose of humic acid a chivied high percentage of change (36.6). Increases in P content in cow pea with vydate treatment were observed to be higher than both of fulvic and humic in cow pea plants. Generally, the contents of tomato and cow pea plants in N, P and K were increased and produced healthy plants than infected plants by *M. incognita.*

Data in Table (6) evaluated the possible relationships existing between humic substances (HA and FA acids) on the nematode reduction. The correlation coefficients were calculated between humic acid properties and the average of numbers variation of galls, pf /pi and percentage of nematode reduction at the various sampling. Results obtained for nematode reduction (Table 3) indicated the occurrence of: significant positive correlations between total acidity, COOH group and total phenolic groups content in fulvic acid than humic acid. Nematode galls, pf /pi and percentage of nematode were redacted *in vivo*. Humic acid content from C, N, S, P percentage was greater than fulvic acid in which, humic acid increased the fresh and dry weight of plants (tomato and cow pea).

DISCUSSION

Humic substances are thought to consist of a skeleton of alkyl or aromatic units cross-linked mainly by oxygen and nitrogen groups, with the major functional groups being carboxylic acid, phenolic and alcoholic hydroxyls, ketone, and quinone groups (Schulten *et al*., 1991). However humic acid showed no effect on survival of J2 of *M. hapla* after 48 h (EL-Miligy and Norton, 1973) and had a significant effect on nematode reproduction on banana (Daneel *et al*., 2000). These results agreed to a great extent with the present results, which showed that humic acid alone had the least effect on *M. incognita* hatching, J2 survival and reproduction on tomato. In the present study humic acid supplemented with Fe, Mn, Cu gave the best results on both *in vitro* and *in vivo*. This treatment gave the greatest reductions in hatching, J2 survival and root penetration, and reproduction of *M. incognitaon* tomato. Such effects were probably due to the present of heavy metals rather than to humic acid. The influence of mineral nutrition on nematode activity has been studied extensively. It has been reported that mineral nutrition plays a vital role in biological and physiological activities in plants, and it can results in an increase or decrease in resistance or tolerance of plants to nematode infection (Siddiqui *et al*., 1999 and El- Naggar, 2001). 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 increase may be involved in synthesizing bioactive compounds able to oppose nematode development and reproduction. 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 (Kang *et al*., 1981 and Wade and Sanchez, 1983). The present results showed that the application of humic acid alone or supplemented with NPK or heavy metals improved significantly plant growth. The best results were attributed to the NPK and heavy metals, which promote root growth, improve uptake of nutrients and also reduce nematode populations (Khan and Khan, 1995), but soil type must be taken into consideration. Similar results were obtained by Singh and Chaudhury (1974) on tomato and Kesba and El- sayed (2005) on grape, whilst contradictory results were reported by Oteifa and El- Gindi (1962) on tomato and Shafee and Jenkins (1963) on pepper. The contradiction of elements, method of application, nematode species and / or populations, or environmental factors during the experimentation most be taken in consideration.

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تـأثير أحمـاض الهيوميـك والفالفيـك علـى الحـد مـن اصـابة نباتـات الطمـاطم ولوبيـا العلف بنيماتودا تعقد الجذور *Meledogyne incognita*

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تم استخلاص كلا من احماض الهيوميك والفالفيك من الكمبوست (مخلف نباتى) وسماد البيوجاز بطريقة الاستخلاص بالقلوى (صودا كاويه ٥ و ع وبوتاسا كاويه ١ع) وتم اختبار كلا المستخلصين على نيماتودا تعقد الجذور *M. incognita* معمليا وتجربة أصص) وذلك على نباتى الطماطم صنف كاسل روك وعلى لوبيا العلف وتم مقارنة تأثيرهما بمبيد نيماتودى (الفايديت ١٠%) وكانت الجرعات المستعمله جرعة أحادية (١ مللى) وجرعة ثنائيه (٢ مللى) من كلا المستخلصين والفايديت).

معمليا حقق المبيد النماتودى (الفايديت) أحسن معدل معنويا فى تثبيط فقس بويضات هذا النوع من النيماتودا عن كلا من الهيوميك والفالفيك على التوالى . كانت نسبة التثبيط ٤٧,٣% مقارنا بالهيوميك و ٤٥,٧% مقارنا بالفالفيك . بينما كان الهيوميك المستخلص من سماد البيوجاز بطريقة الصودا كان أخر معاملة فى التأثير على نسبة فقس البيض) ولكن الهيوميك المستخلص من سماد البيوجاز بطريقة البوتاسا حقق أحسن معدل فى تخفيض يرقات هذا النوع من النيماتودا وذلك عند الجرعه (٢ مللى) وكانت نسبة التثبيط ٢٦,٠٩% متفوقا على المبيد النيماتودى .

وعلى النقيض من ذلك حامض الفالفيك المستخلص من الكمبوست بطريقة الصودا كان أقل نسبه تنشيط فى الفقص بينما المستخلص من سماد البيوجاز بطريقة البوتاسا حقق أعلى معدل معنوى فى تخفيض أعداد اليرقات وكان نسبة التثبيط فى اليرقات ٤٥,١% .

فى تجربة أصص) تم أختيار كلا من أحماض الهيوميك والفالفيك المستخلصين من سماد البيوجاز بطريقة البوتاسا وتم عمل عدوى نيماتوديه على كلا من نبات الطماطم واللوبيا وتم استعمال نفس الجرعات السابقة الاحاديه (١ مللى) والثنائيه (٢ مللى).

وكانت النتائج كالتالى : حققت الجرعة الثانية من حامض الفالفيك انخفاضا معنويا فى أعداد galls (التورمات الموجوده على الجذور) والاعداد النهائيه بالنسبه للاعداد الأوليه Pf / pi بالاضافه لنسبة الاختزال فى أعداد النيماتود وتفوق على جرعة الاحاديه وكلا من جرعتين الهيوميك وكان تاثير الجرعة الثانية من حامض الفالفيك قريبا" من تاثير الجرعه الثانيه من المبيد النيماتودى .

كما حققت الجرعه (٢ مللى) من حامض الهيوميك أعلى معدل زياده فى نمو النباتات متمثلا فى الوزن الطازج والجاف . ولم تؤثر كل المعاملات على محتوى النبات من النتروجين الكلى . بينما حققت كلا الجرعتين من الفالفيك زياده فى محتوى الطماطم من الفوسفور الكلى . وحقق المبيد النيماتودى أعلى معدل زياده فى محتوى كلا النباتين من البوتاسيوم الكلى عن كل المعاملات.

Table (5): Effect of humic and fulvic acids on tomato N, P and K content in sandy loam soil

Treatment	Tomato						Cow pea					
	N	Change %	P	Change %	K	Change%	N	Change %	P	Change %	K	Change %
humic 1.0 ml	0.049^{e}	- 31.0	0.24^{cd}	- 17	0.71 ^{cd}	- 21	0.08 ^{e}	- 48.0	0.41^{e}	- 21.2	1.19 ^{a}	+ 36.8
humic 2.0 ml	0.056^{de}	- 21.0	0.14^{d}	- 51.7	0.75 ^{d}	- 16.7	0.08 ^{e}	- 52.0	0.54^{cd}	+ 3.8	1.15 ^{a}	+ 32.2
fulvic 1.0 ml	0.068^{d}	- 4.2	0.68^{a}	+ 134	0.79 ^{a}	- 12.2	0.11 ^{d}	- 34.9	0.47^{de}	- 9.6	1.06 ^{b}	+ 21.8
fulvic 2.0 ml	0.061d^{e}	- 14.0	0.49^{b}	+ 68.9	0.54 ^{b}	- 40	0.11 ^{d}	- 37.0	0.32^{f}	- 38.5	0.99 ^{b}	+ 13.8
vydate1.0 ml	0.093^{c}	+ 31.0	0.48^{b}	+ 65.5	0.93 ^{b}	+ 3.3	0.12 ^{c}	- 27.2	0.59^{bc}	+ 13.5	1.15 ^{a}	+ 32.2
vydate2.0 ml	0.137^{b}	+ 50.7	0.43^{b}	+ 48.3	0.99 ^{b}	+ 10	0.1 ^{d}	- 40.0	0.76^{a}	+ 46.2	1.0 ^{b}	+ 14.9
Haelthy	0.154^{a}	+ 117	0.72^{a}	+ 148	1.05 ^{a}	+ 16.7	0.15 ^{b}	- 9.5	0.63^{b}	+ 21.2	1.15 ^{a}	+ 32.2
Nematode	0.071^{d}	-	0.24^{cd}	-	0.90 ^{c}	-	0.17 ^{a}	-	0.52^{cd}	-	0.87 ^{c}	-
LSD	0.0142		0.113		0.142		0.0113		0.0711		0.0781	

- **Means followed by the same letter (s) within a column in each block are not significantly different ($p \leq 0.05$) according to duncans multiple range test.**

Table (6): Characteristic of humic and fulvic acids extracted from biogas manure

Treatment		C %	N %	H %	S %	O %	P ppm	K ppm	Total acidity (mmol / 100g)	CooH groups (mmol / 100g)	Phenolic groups (mmol / 100g)
KOH 1.0 N	HA Bio.m	51.7	2.27	5.07	9.60	31.36	0.001	0.017	275	210	65
	FA Bio.m	30.6	0.30	34.99	1.02	33.09	0.016	0.240	600	300	300

- HA Bio.m: Humic acid extracted from Biogas FA Bio.m: Fulvicacid extracted from Biogas Manure.