EFFECT OF VOLATILE FATTY ACIDS ON EMBRYOGENESIS AND HATCHING OF *MELOIDOGYNE INCOGNITA* EGGS

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Summary. *Meloidogyne incognita* egg masses were immersed in different concentrations of six volatile fatty acids to bioassay their toxic effect on embryonic development and hatching. Exposure to a 250 ppm aqueous solution of volatile fatty acids completely suppressed egg hatching within 48 hours. When exposed to a 125 ppm concentration hatching was reduced 75% by acetic acid, 91% by propionic acid, 23% by butyric acid, 63% by isobutyric acid, 41% by valeric acid and 69% by caprylic acid during 12 days of incubation. In revival studies hatching did not resume in acetic and caprylic acid-treated eggs even after rinsing in water, whereas it resumed in isobutyric and propionic acid treated eggs. Valeric acid delayed hatching. Results indicated that acetic and caprylic acids reduced hatching most effectively and irreversibly by impairing embryogenesis at 'static-vermiform' and 'gastrula' stages respectively.

A variety of organic amendments have been shown to decrease nematode infestations in the soil ecosystem (Sikora, 1992). On incorporation the organic matter is acted upon by native soil microflora and as a consequence of the enhanced microbial activity, antibiosis towards nematode activity is initiated. This is due to the accumulation of toxic metabolites which are either produced during microbial growth (Djian *et al.,* 1991) or released as decomposition products during microbial breakdown of the organic matter (Sayre *et al.,* 1965). Several short chain volatile fatty acids excreted or released during microbial decomposition of organic matter, as pointed out by Badra *et al.* (1979), were identified gas chromatographically as formic, acetic, propionic, butyric and valeric acids (Castillo, 1985). Although fatty acids are known to be toxic to various developmental stages of plant parasitic nematodes (Djian *et al.,* 1991), their effect at different concentrations on nematode activity is unknown. Such information would help to screen the candidature and the biocontrol potential of various bacteria known to produce these organic acids.

This paper reports on a study to determine the effect of volatile fatty acids on embryonic development and hatching of *Meloidogyne incognita* eggs.

MATERIALS AND METHODS

Aqueous stock solutions of five volatile fatty acids (VFAs) namely acetic (CH_3COOH), propionic (CH_3CH_2COOH), butyric ($CH_3CH_2CH_2COOH$), isobutyric [$(CH_3)_2$ $CHCOOH$] and valeric ($C_5H_{10}O_2$) acids were prepared in double distilled water; caprylic [$CH_3(CH_2)_6COOH$] acid was emulsified with 0.1 per cent Tween-80. All stocks were stored at 5 °C and standard solutions were prepared by serial dilutions to give a final concentration of 125, 250 and 500 ppm of each acid. To bioassay the toxicity of VFAs on nematode egg hatching, the mature egg masses of *Meloidogyne incognita* (Kofoid *et* White) Chitw. were collected from tomato roots seven weeks after inoculation. Batches of egg masses were placed on fine nylon mesh in solid watch glasses containing 5 ml freshly prepared VFA solution and covered and incubated at 28 °C. Emerging juveniles were counted at 1, 2, 4, 6, 8, 10 and 12 days. Fresh solutions were replenished after each observation. Distilled water was taken as control. After the 12th day of incubation the egg masses were washed free of VFA solution and further hatching was allowed in distilled water for another seven days to observe resumption of hatching. Each treatment was replicated three times.

To study the effect of VFA solutions on embryonic development, the eggs remaining unhatched after 19 days of incubation were treated with sodium hypochlorite solution and unhatched eggs counted. Approximately one hundred eggs from each treatment were examined microscopically and grouped into the following three embryonic development stages: 1. blastula-undifferentiated multicellular, 2. gastrula-differentiated mass of cells along with vacuoles, 3. vermiform fully-organised, coiled juvenile. Stage three was further subdivided based on juvenile movement within the egg i.e. 3a static non-motile and 3b motile juvenile.

For the calculation of per cent hatch average number of eggs per egg mass of the respective treatment was considered. Analysis of variance was run on the transformed data (i.e. square root and arsine transformation wherever necessary) to compare the means of different VFAs using Duncan's Multiple Range Test as described by Snedecor and Cochran (1968). Correlation coefficient between concentration of VFAs with per cent hatch at different time intervals was also computed.

RESULTS AND DISCUSSION

In general, exposure of *M. incognita* eggs to different VFA solutions suppressed nematode hatch (Table I). Exposure to a 250 ppm concentration or more completely suppressed egg hatching within 48 hours. Correlation coefficients between concentration of VFAs and percent hatch at different time intervals revealed a highly negative association, suggesting percent hatch decreased with increase in concentration of VFAs (Table II). Comparing nematicidal activity, propionic and acetic acids were most effective at the lowest concentration (125 ppm) tested and the percentage of emerging juveniles decreased progressively with time of incubation. Their cumulative percent of hatch was 9.4 and 25, respectively, after twelve days of incubation compared to 87.7 percent in water (Table I).

Hatching was 77 percent in butyric acid but only 36.7 percent in isobutyric acid which is an isomer of the butyric acid. Large differences in the nematicidal efficacy between butyric and isobutyric acids at equimolar concentration indicate that the nematotoxic property of VFAs is not related exclusively to pH. This is further evident from the fact that acetic acid, which is a comparatively more acidic and ionisable molecule than propionic acid, showed a less nematotoxic effect than the latter. Our observations agree with a previous report (Djian *et al.*, 1991) that toxicity of VFA is vested in the undissociated form and not in their radicals. The glycoproteins forming the nematode surface coat adsorbs ionic molecules, thus epicuticle and epidermal cell membrane allow entry of unionised form of VFA (Marks *et al.*, 1968) which are involved in nematoxicity.

When the treated egg masses were rinsed in water after 12 days of incubation, hatching failed to resume in acetic and caprylic acid treated eggs even after incubation for another seven days in water (Table I), suggesting their irreversible toxic effect on embryogenesis. However, hatching resumed immediately and at a rate greater than in the control with 42% and 34% revival of eggs in propionic and isobutyric acid treatments, respectively (Table I). This indicates that embryogenesis proceeded normally and that the contact effect of these VFAs might be just sufficient to disrupt the complex behavioural sequence preceding eclosion. Valeric acid, however, did not differ significantly from the control as it suppressed up to 89 percent hatching (Table I) but with a reduced rate of hatch per unit time and thus exhibited a delaying effect on hatching. At the end of the incubation period of 19 days both in VFAs and water, acetic and caprylic acids were found most effective. They irreversibly reduced larval emergence by 72.9 and 66.5 percent, respectively. Propionic followed by inhibiting eclosion by 48.6 percent. The least toxicity of butyric acid seems obvious owing to its metabolic requirement for the synthesis of g-aminobutyric acid (GABA), a neurotransmitter used by motor-neurons in nematodes (Johnson and Stretton, 1987). Like-wise the greater toxicity of isobutyric acid can be explained on the basis of its metabolic utilisation as a 'substrate-analogue' of butyric acid because of their molecular similarity. Therefore, its action as a 'competitive-inhibitor' can be speculated. Selective nematotoxic effect of VFAs on plant parasitic nematodes and not on saprophagous, entomophagous or animal parasites (Djian *et al.,* 1991) has also been attributed to their evolution in and acclimatization to the environment that abounded in these acids i.e. decomposing plant residue (Sayre *et al.,* 1965) while plant parasitic species were not exposed to such environments.

Microscopic examination of the VFAs treated eggs remaining unhatched after 19 days of incubation revealed that different VFAs affected embryogenesis at different stages (Table III). Caprylic acid stopped development beyond the 'gastrula' stage, while acetic, propionic and valeric acids allowed most of the eggs to reach 'static-vermiform' stage and did not allow further development into the moving stage, which is necessary for hatching. In the butyric and isobutyric treated eggs 'moving vermiform' stage was also recorded in 34.4 and 36.7% eggs, respectively. From these studies it is evident that caprylic and acetic acids irreversibly impaired embryogenesis at gastrula and static vermiform stages, respectively. These observations indicated that advanced stages of embryonic development were sensitive to VFAs and thus a neuromuscular correlation can be anticipated to explain stage specificity of VFAs. The paralysing effect (Bansal *et al.*, 1998) and disruption in the movement of infective juveniles of *Meloidogyne* sp. due to acetic acid observed by Djian *et al.* (1991) suggest that lower concentrations of fatty acids might affect the behavioural sequence necessary for host root invasion.

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Table I. Mean percentage of juveniles of *Meloidogyne incognita* hatched per egg mass during incubation in volatile fatty acids.

VFA	Conc. (ppm)	Incubation in VFA (days)								Incubation in water (days)					G. Total (A+B)
		1	2	4	6	8	10	12	Total (A)	1	3	5	7	Total (B)	
Acetic acid	125	6.20^{b}	7.60^{b}	1.00^{d}	0.50^{d}	2.00^{c}	1.50^{c}	6.20^{cd}	25.00^{e}	0.50^{e}	1.00^{c}	0.60^{c}	0.00^{b}	2.10^{d}	27.10^{d}
	250	2.90^{b}	1.00^{bc}	0.40^{c}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{b}	4.30^{cd}	0.00^{d}	1.40^{d}	0.70^{b}	1.80^{c}	3.90^{e}	8.20^{e}
	500	1.50^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	1.50^{b}	0.00^{c}	0.40^{bc}	0.00^{c}	0.00^{b}	0.40^{d}	1.90^{d}
Propionic acid	125	6.80^{b}	0.70^{d}	0.70^{d}	0.30^{d}	0.30^{c}	0.30^{C}	0.30^{e}	9.40^{f}	9.70^{b}	14.90^{a}	11.30^{a}	6.10^{a}	42.00^{a}	51.40^{c}
	250	1.40^{c}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{b}	0.00^{b}	1.20^{b}	2.60^{d}	7.70^{a}	14.00^{c}	1.90^{b}	0.00^{c}	23.60^{d}	26.20^{d}
	500	0.90^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.90^{b}	1.00^{bc}	0.00^{c}	0.00^{c}	0.40^{b}	1.40^{cd}	2.30^{d}
Butyric acid	125	11.70^{a}	7.30^{b}	17.80^{a}	4.70^{c}	8.60^{b}	15.90^{a}	11.00^{bc}	77.00^{b}	2.10^{de}	6.50^{b}	6.00^{b}	0.40^{b}	15.00^{c}	92.00^{a}
	250	2.10^{bc}	0.00^{c}	0.00^{c}	1.40^{c}	0.70^{b}	0.70^{b}	2.10^{b}	7.00^{C}	9.40^{a}	22.00^{b}	24.80^{a}	4.20^{b}	60.40^{b}	67.40^{ab}
	500	0.60^{bc}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.60^{b}	0.00^{c}	2.20^{b}	7.30^{a}	10.70^{a}	20.20^{b}	20.80^{c}
Isobutyric acid	125	14.10^{a}	14.60^{a}	1.30^{d}	0.00^{d}	0.90^{c}	1.30^{c}	4.50^{de}	36.70^{d}	16.80^{a}	16.40^{a}	0.40^{c}	0.40^{b}	34.00^{b}	70.70^{b}
	250	2.50^{bc}	1.10^{bc}	0.80^{c}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{b}	4.40^{cd}	3.30^{c}	49.50^{a}	24.00^{a}	0.30^{c}	77.10^{a}	81.50^{ab}
	500	0.90^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.90^{b}	1.00^{bc}	1.40^{bc}	1.07^{b}	0.40^{b}	3.80^{cd}	4.70^{d}
Valeric acid	125	6.10^{b}	5.50^{bc}	10.20^{b}	11.90^{a}	1.10^{c}	9.70^{b}	14.40^{ab}	58.90^{c}	5.50^{c}	8.30^{b}	12.40^{a}	3.90^{a}	30.10^{b}	89.00^{a}
	250	2.10^{bc}	0.30^{bc}	0.30^{c}	0.00^{d}	0.70^{b}	1.80^{b}	0.90^{b}	6.10^{c}	5.40^{b}	14.30^{c}	8.90^{b}	8.70^{a}	37.30^{c}	43.40^{c}
	500	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{b}	0.40^{b}	0.80^{b}	0.80^{b}	2.00^{b}	14.10^{a}	19.60^{a}	7.10^{a}	0.40^{b}	41.20^{a}	43.20^{b}
Caprylic acid	125	3.70^{bc}	4.60^{c}	4.20^{c}	10.90^{ab}	0.80^{c}	2.10^{c}	5.10^{de}	31.40^{de}	1.20^{de}	0.90^{c}	0.00^{c}	0.00^{b}	2.10^{d}	33.50^{d}
	250	4.40^{a}	2.20^{b}	9.50^{b}	4.40^{b}	0.00^{b}	0.00^{b}	0.00^{b}	20.50^{b}	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{e}	20.50^{de}
	500	0.4^{cd}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.4^{b}	0.0^{c}	0.0^{c}	0.0^{b}	0.0^{d}	0.0^{d}	0.4^{d}
Control		1.80^{c}	6.90^{bc}	12.80^{b}	9.10^{b}	24.40^{a}	14.70^{a}	18.00^{a}	87.70^{a}	2.80^{d}	1.13^{c}	1.20^{c}	0.50^{b}	5.70^{d}	93.40^{a}

Means with different superscripts in a column differ significantly ($P<0.05$) for respective concentration.

Table II. Correlation coefficients between concentration of volatile fatty acids and percent hatch at different time intervals.

VFA	Incubation in VFA (days)								Incubation in water (days)					G. Total (A+B)
	1	2	4	6	8	10	12	Total (A)	1	3	5	7	Total (B)	
Acetic acid	-0.85**	-0.82**	-0.76*	-0.59^{NS}	-0.68*	-0.70*	-0.63^{NS}	-0.80**	-0.74*	-0.63^{NS}	-0.62^{NS}	-0.18^{NS}	-0.61^{NS}	-0.87**
Propionic acid	-0.79*	-0.69*	-0.72*	-0.53^{NS}	-0.71*	-0.53^{NS}	-0.36^{NS}	-0.86**	-0.93**	-0.93**	-0.78*	-0.62^{NS}	-0.98**	-0.97**
Butyric acid	-0.79**	-0.74*	-0.75*	-0.89**	-0.78*	-0.75*	-0.84**	-0.79*	-0.39^{NS}	-0.38^{NS}	-0.12^{NS}	+0.94**	-0.08^{NS}	-0.95**
Isobutyric acid	-0.78*	-0.79*	-0.94**	-	-0.71*	-0.74	-0.60^{NS}	-0.80**	-0.82**	-0.47^{NS}	-0.15^{NS}	+0.03^{NS}	-0.55^{NS}	-0.87**
Valeric acid	-0.91**	-0.78*	-0.77*	-0.74^{1}	-0.78*	-0.81**	-0.75*	-0.79*	+0.85**	+0.92**	-0.84**	-0.55^{NS}	+0.72*	-0.74*
Caprylic acid	-0.83*8	-0.95**	-0.58^{NS}	-0.92**	-0.75*	-0.74*	-0.75*	-0.98**	-0.69*	-0.75*	-	-	-0.72*	-0.98**

* Significant at 5% level.
** Significant at 1% level.

Table III. Effect of volatile fatty acids on embryonic development stages of *M. incognita* eggs.

	Treatment/ conc. (ppm)	Blastula	Gastrula	Static	Moving
125	Acetic acid	$19.3^{b}\pm1.21$	$17.8^{f}\pm0.98$	$54.3^{c}\pm1.44$	$8.6^{c}\pm0.75$
	Propionic acid	$5.2^{e}\pm0.11$	$33.1^{c}\pm0.86$	$59.0^{b}\pm0.11$	$2.7^{d}\pm0.63$
	Butyric acid	$3.4^{e}\pm0.34$	$6.9^{f}\pm0.05$	$55.3^{c}\pm0.15$	$34.4^{b}\pm0.43$
	Isobutyric	$10.9^{c}\pm0.98$	$21.1^{d}\pm0.69$	$31.3^{e}\pm0.23$	$36.7^{a}\pm1.44$
	Valeric acid	$7.7^{d}\pm0.46$	$20.5^{d}\pm0.51$	$64.1^{a}\pm0.63$	$7.7^{c}\pm0.69$
	Caprylic acid	$29.4^{a}\pm0.92$	$66.2^{a}\pm0.71$	$3.7^{f}\pm0.63$	$0.60^{f}\pm0.48$
	Control (Water)	$0.1^{f}\pm0.00$	$50.6^{b}\pm0.91$	$45.6^{d}\pm0.35$	$4.0^{d}\pm0.57$
250	Acetic acid	$10.6^{b}\pm0.34$	$25.1^{c}\pm0.98$	$60.8^{c}\pm0.17$	$3.5^{e}\pm0.46$
	Propionic acid	$10.0^{b}\pm0.23$	$7.0^{g}\pm0.69$	$76.9^{a}\pm1.03$	$6.1^{d}\pm0.57$
	Butyric acid	$8.5^{c}\pm0.78$	$21.2^{d}\pm1.00$	$56.4^{d}\pm1.21$	$13.9^{c}\pm0.95$
	Isobutyric	$5.9^{d}\pm0.63$	$17.6^{e}\pm1.03$	$52.9^{e}\pm0.23$	$23.6^{a}\pm1.44$
	Valeric acid	$0.2^{e}\pm0.05$	$14.6^{f}\pm0.46$	$66.4^{b}\pm0.57$	$18.8^{b}\pm0.05$
	Caprylic acid	$18.2^{a}\pm0.23$	$78.9^{a}\pm0.80$	$2.6^{g}\pm0.46$	$0.3^{f}\pm0.11$
	Control (Water)	$0.1^{e}\pm0.00$	$50.6^{b}\pm0.91$	$45.6^{f}\pm0.35$	$4.0^{de}\pm0.57$
500	Acetic acid	$4.2^{b}\pm0.37$	$8.4^{e}\pm0.11$	$87.1^{a}\pm0.28$	$0.1^{c}\pm0.05$
	Propionic acid	$3.9^{b}\pm0.34$	$7.3^{e}\pm0.40$	$77.9^{b}\pm1.21$	$10.9^{a}\pm0.46$
	Butyric acid	$0.4^{c}\pm0.11$	$27.5^{d}\pm0.89$	$66.6^{c}\pm0.11$	$5.5^{b}\pm0.89$
	Isobutyric acid	$9.5^{a}\pm0.40$	$38.2^{c}\pm0.51$	$42.7^{f}\pm0.34$	$9.6^{a}\pm0.57$
	Valeric acid	$0.1^{c}\pm0.00$	$39.7^{c}\pm1.03$	$55.1^{d}\pm1.21$	$5.1^{b}\pm0.17$
	Caprylic acid	$0.1^{c}\pm0.05$	$83.3^{a}\pm1.15$	$15.9^{g}\pm0.75$	$0.3^{c}\pm0.17$
	Control (Water)	0.1 ± 0.00^{c}	$50.6^{b}\pm0.91$	$45.6^{e}\pm0.35$	$4.0^{b}\pm0.57$

Means bearing different superscripts in a column differ significantly ($P<0.05$) at each concentration.

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