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IN VITRO HATCHING OF ROOT-KNOT NEMATODE (*Meloidogyne incognita*) EGGS AFTER A BRIEF EXPOSURE TO OKRA ROOT EXUDATES [1]

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In the *Meloidogyne*, the evidence for stimulation of hatching is rather scanty and often conflicting (*2*); however, where increased larval hatching has been observed *in vitro*, it was low and significant (*3*). Significantly less number of eggs of *M. javanica* hatched in nonsterile soil than in sterile soil (*5*). Tomato seedlings grown in the nonsterile soil counteracted the inhibition of hatching, probably caused by the soil microganisms, and only slightly more larva hatched in the sterile soil. However, addition of tomato root exudates to the nonsterile soil did not prevent the inhibition of larval hatching. Little information is available about the *in vitro* hactching process in the *M. incognita*. This communication provides information on the effect of a brief exposure to the root exudates of okra on the hatching of *M. incognita* eggs.

Methods and Materials. Root exudates were collected aseptically from seedlings of okra (*Abelmoschus esculentus* cv. Pusa Sawani) germinated in 250 ml conical flasks containing three layers of Whatman paper N.º 1 at the bottom (*4*). Flasks (growth chambers) were incubated in the culture room at 25 ± 2ºC. Hoagland nutrient culturing solution was added every 48 hours so as to keep the filter paper wet. After 3 weeks of seedling growth, flasks were rinsed twice with 50 ml of sterile distilled water, and the solution thus obtained was stored at 5ºC. Filter papers were cut into small shreds and transferred to a flask containing 50 ml of sterile distilled water and placed on a shaker for 30 min. Exudate from filter papers was combined with rinsing solution, concentrated *in vitro* and dried in a vacuum dessicator. Then, it was passed through a sterile filter paper which had been moistened with 90 per cent methylalcohol and dried before hand, and was used for experimental purposes. Stock solutions of root exudates were stored at 5ºC (*1*).

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Four egg masses of uniform size (an egg mass containing approx. 400 eggs), surface sterilized (using Hg Cl_2 0.1%), were placed in cavity blocks containing 0.5 ml of okra root exudates or distilled water. Three different experiments were carried out involving exposure of the eggs to root exudates:

a) Egg masses in okra root exudates for 96 hours
b) Egg masses in okra root exudates for 15 min. and then in sterile distilled water for 96 hours.
c) Egg masses in the sterile distilled water (control) for 96 hours. Larval hatching was recorded 24 hours after the 96-hour period in the three different experiments, each having five replicates. Fresh root exudate was added at 24-hour intervals from the stock solution after rinsing with sterile distilled water; thus, the egg masses in all three experiments were exposed to the mechanical stimulation and oxygenation with rinsing. The hatched larvae from the cavity blocks were pipetted out, and counted for determining the percentage hatch over the period. This ensured that any differences in hatch between experiments were due solely to differences in root exudate treatment. Results were analysed using two-way analysis of variance after arcsin transformation of percentages.

Results and Discussion. The observations on the *in vitro* hatching of root-knot nematode (*M. incognita*) are presented in Fig. 1. An egg exposure time of 15 minutes to okra root exudate induced a 11% hatch of larvae after 96 hours. This hatch was significantly different from both that in sterile distilled water (6%, $p < 0.01$) and from those eggs which were continuously in okra root exudates (19 %, $p < 0.05$). The increase in egg hatch was induced by the brief exposure to root exudates and not due to the remaining traces of hatching factor because washing for 15-20 min. almost completely removed the hatching stimulus (1). Present investigations are in keeping with those recorded for *Globodera pallida* (*1, 2*). No further egg hatching was noted in the batches of egg masses placed in these washings; or, no differences from the control.

Furthermore, the 15-20 min. washing did not increase the level of egg hatching from egg masses previously exposed to the sterile distilled water. Exposure of eggs to the root exudate triggered the hatching response (*1, 4*).

It is known that the egg shell is permeable to the hatching factor which quickly enters into the eggs (*3, 4*). However, it has not been known, until now, just how rapidly root exudates act upon the eggs to initiate the sequence of events resulting in the hatch of larvae.

RESUMO

(ECLOSÃO «IN VITRO» DE LARVAS DE *Meloidogyne incognita* APÓS BREVE EXPOSIÇÃO A EXSUDATOS DE RAÍZES DE QUIABO)

Massas de ovos de *Meloidogyne incognita* foram expostas a exsudatos de raízes de quiabo por 15 minutos e, depois, mantidas em água destilada estéril por 96 horas. A porcentagem de eclosão das larvas foi, então, calculada e comparada aos resultados dos tratamentos em que se mantiveram as massas de ovos no exsudato ou somente em água destilada estéril durante 96 horas. A eclosão das larvas nesses três tratamentos foi de 11%, 19% e 6%, respectivamente. Já é reconhecido que a casca dos ovos de nematóides é permeável a fatores de eclosão, que penetram rapidamente no ovo. Contudo, não se sabia ainda, com exatidão, qual seria o tempo mínimo necessário para que exsudatos de raízes agissem sobre os ovos, dando início à seqüência de eventos que resultam na eclosão das larvas.

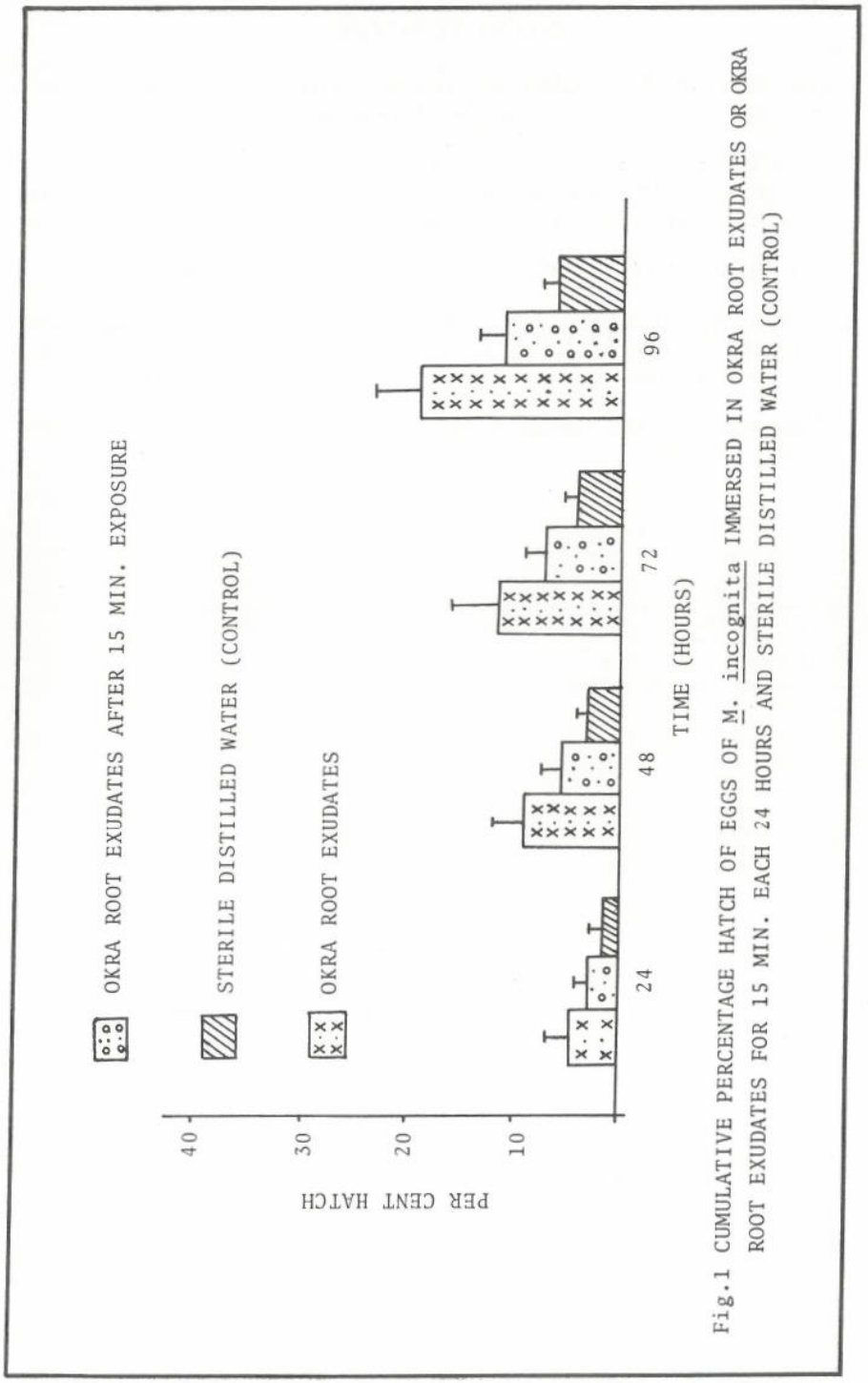

Fig.1 CUMULATIVE PERCENTAGE HATCH OF EGGS OF M. incognita IMMERSED IN OKRA ROOT EXUDATES OR OKRA ROOT EXUDATES FOR 15 MIN. EACH 24 HOURS AND STERILE DISTILLED WATER (CONTROL)

CITED LITERATURE

1. FORREST, J.M.W. & PERRY, R.N. Hatching of *Globodera pallida* eggs after brief exposure to potato root diffusate. *Nematologica 26*:130-32. 1980.

2. SHEPHERD, A.M. & CLARKE, A.J. Molting and hatching stimuli. *In* Zuckerman, B.M., Mai, W.F. & Rohde, R.A. (cd.). *Plant Parasitic Nematodes*. Vol. II. N. York, Academic Press, 1971. p. 267-287.

3. SOUTHEY, J.F. *Plant Nematology*. London, H.M. S.O., 1978. 440 p.

4. TANDA, A.S. *Studies on the nature of root exudates of sesame (Sesamum orientale) and its effect against the root-knot nematode (Meloidogyme incognita) in okra*. Ludhiana, Punjab Agric. Univ., 92 p. 1978. (Ph. D. dissertation)

5. WALLACE, H.R. *The Biology of Plant Parasitic Nematodes*. London, Edward Arnold, 1963. 280 p.