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Nematicidal effect on post-embryonic processes of *Cruznema tripartitum* (Nematoda)

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Abstract. The effect of aldicarb, carbofuran and phorate at concentrations of 25, 50, 75, 100, 125 and 150 ppm was studied on freshly hatched juveniles of *Cruznema tripartitum*. The nematicides prolonged post-embryonic development but reduced the fecundity and reproductive period by affecting the moulting and egg laying processes. The survival time was also reduced in the treated specimens. No intra-uterine development was observed in the adults developed from the treated juveniles. In separate experiments, when fertilised young (2-3 days) and old (7-8 days) females were exposed to the same set of nematicides, the older females showed a strong tendency of retention of eggs. However, the majority of such eggs were non-viable and did not develop.

Keywords. *Cruznema tripartitum*, intra-uterine development, fecundity, nematicides, post-embryogenesis, reproductive period, survival, toxicity.

INTRODUCTION

In continuation with the studies made on *Cephalobus persegnis* (Tahseen *et al.*, 1996a) and *Mesorhabditis cranganorensis* (Tahseen *et al.*, 1996b) revealing the impact of nematicides on embryogenesis and reproductive biology, the present paper highlights the nematicidal impact on the post-embryonic processes of *Cruznema tripartitum*, an amphimictic rhabditid nematode showing a high incidence of intra-uterine development.

MATERIALS AND METHODS

The nematodes were collected from sewage slurry of the Zoology Department, Aligarh Muslim University, Aligarh. They were extracted by sieving and decantation, and modified Baermann's funnel techniques. One freshly moulted adult female and one male were cultured monoxenically in 1.5% water agar with *E. coli* as diet and the progeny was obtained for study. The carbamate nematicides, viz., aldicarb (10%), carbofuran (5%) and the organophosphate nematicide phorate (25%) were used in concentrations of 25, 50, 75, 100, 125 and 150 ppm (200 ppm instantly killed the nematodes). For experiments each nematicide solution was mixed with an equal volume of molten water agar in Petri dishes. Twenty freshly hatched juveniles of *Cruznema tripartitum* were treated with different concentrations of nematicides to study their impacts on post-embryogenesis, moulting, fecundity, reproductive period and total survival time on the females of *C. tripartitum*. In separate experiments, twenty young (1-2 days) and old (7-8 days) fertilised females of *C. tripartitum* were exposed to different concentrations of nematicides and observations were made on their fecundity, reproductive period and intra-uterine development. The experiments were replicated five times and the control experiments were conducted on the untreated nematodes. All the observations were made at 28±2°C temperature.

RESULTS AND DISCUSSION

Post-embryogenesis (Fig. 1)

The freshly hatched juveniles successfully completed post-embryonic development in 25 and 50 ppm conc. of all nematicides though the duration of successive stages increased as compared to control (Fig. 1, A,B). The first stage juveniles treated with 75 ppm carbofuran and phorate (Fig. 1, C) moulted into second stage after 41 h and 49 h, respectively, but failed to do so in aldicarb in which they died after 55 h. No development occurred at 100, 125 and 150 ppm concentrations. The sex ratio which was 1:1 under control conditions shifted to 2:3 with a higher incidence of males under treated conditions.

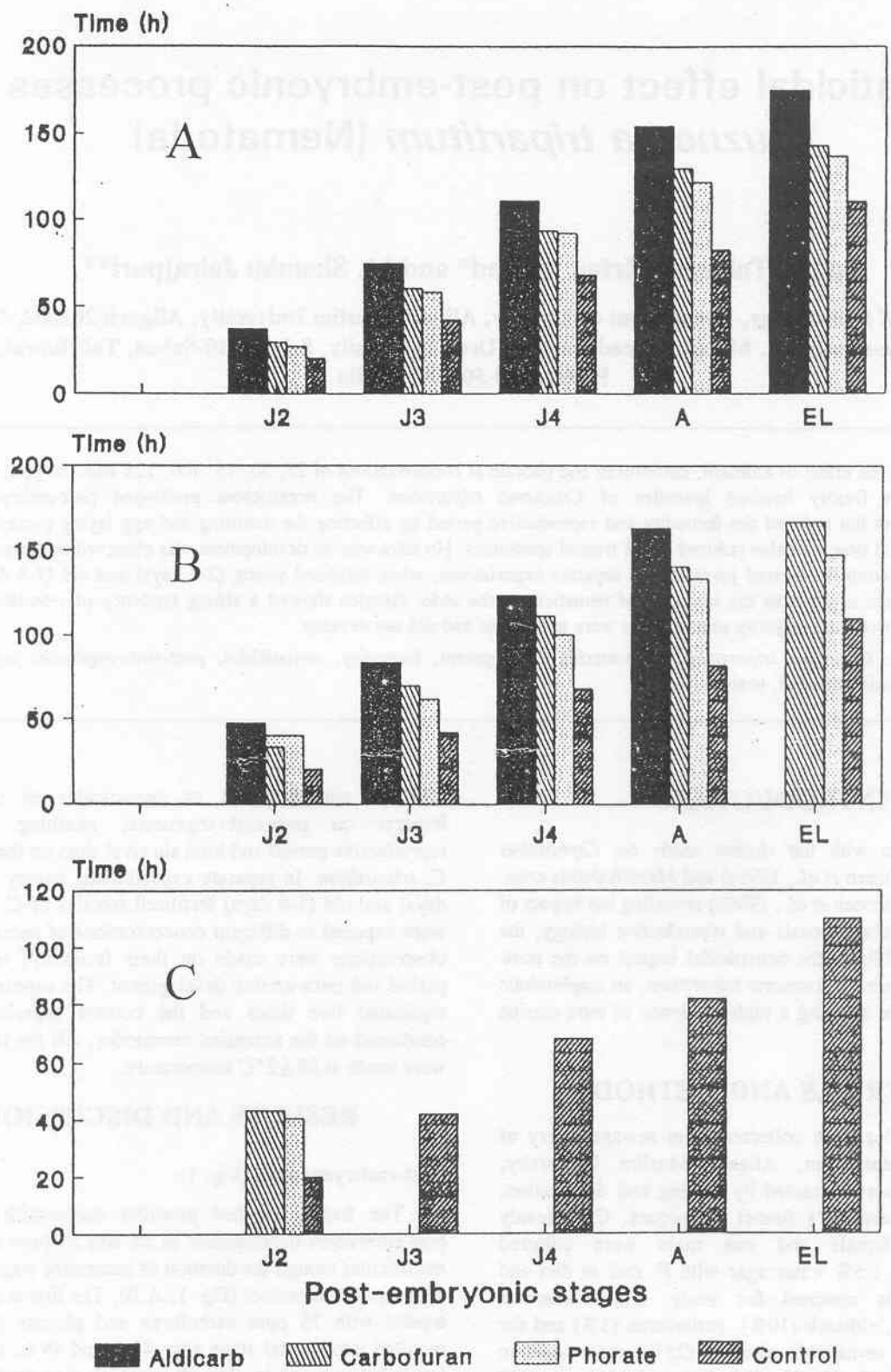

Fig. 1. Nematicidal effect on the post-embryogenesis period of *Cruznema tripartitum*. A: at 25 ppm concentration; B: at 50 ppm concentration; C: at 75 ppm concentration.

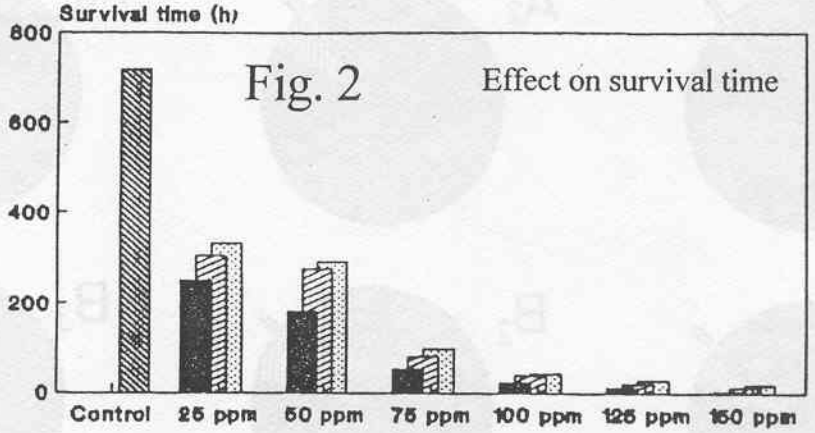

Fig. 2 Effect on survival time

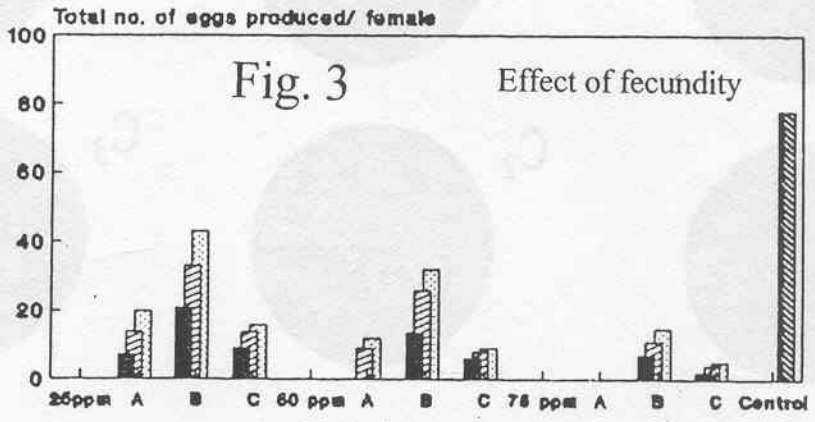

Fig. 3 Effect of fecundity

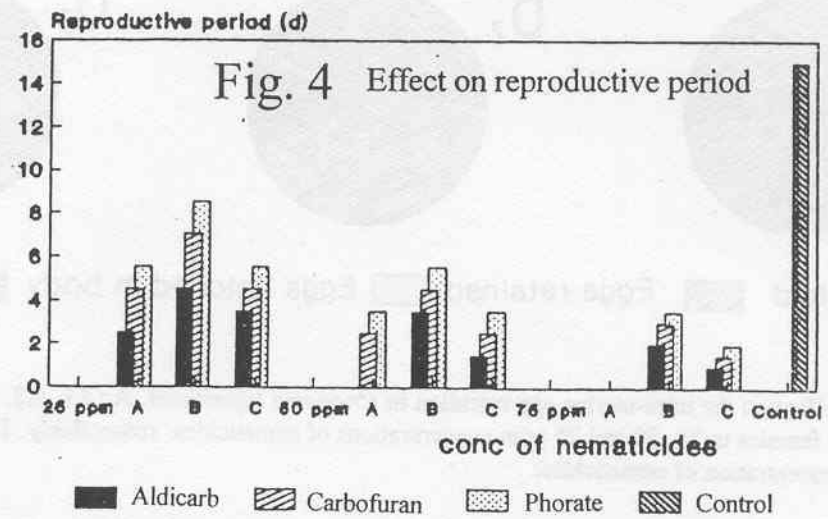

Fig. 4 Effect on reproductive period

A- females developed from treated J1. B- younger females. C- older females

Fig. 2. Nematicidal effect on the survival time of *Cruznema tripartitum*.

Fig. 3. Nematicidal effect on the fecundity of *Cruznema tripartitum*.

Fig. 4. Nematicidal effect on the reproductive period of *Cruznema tripartitum*.

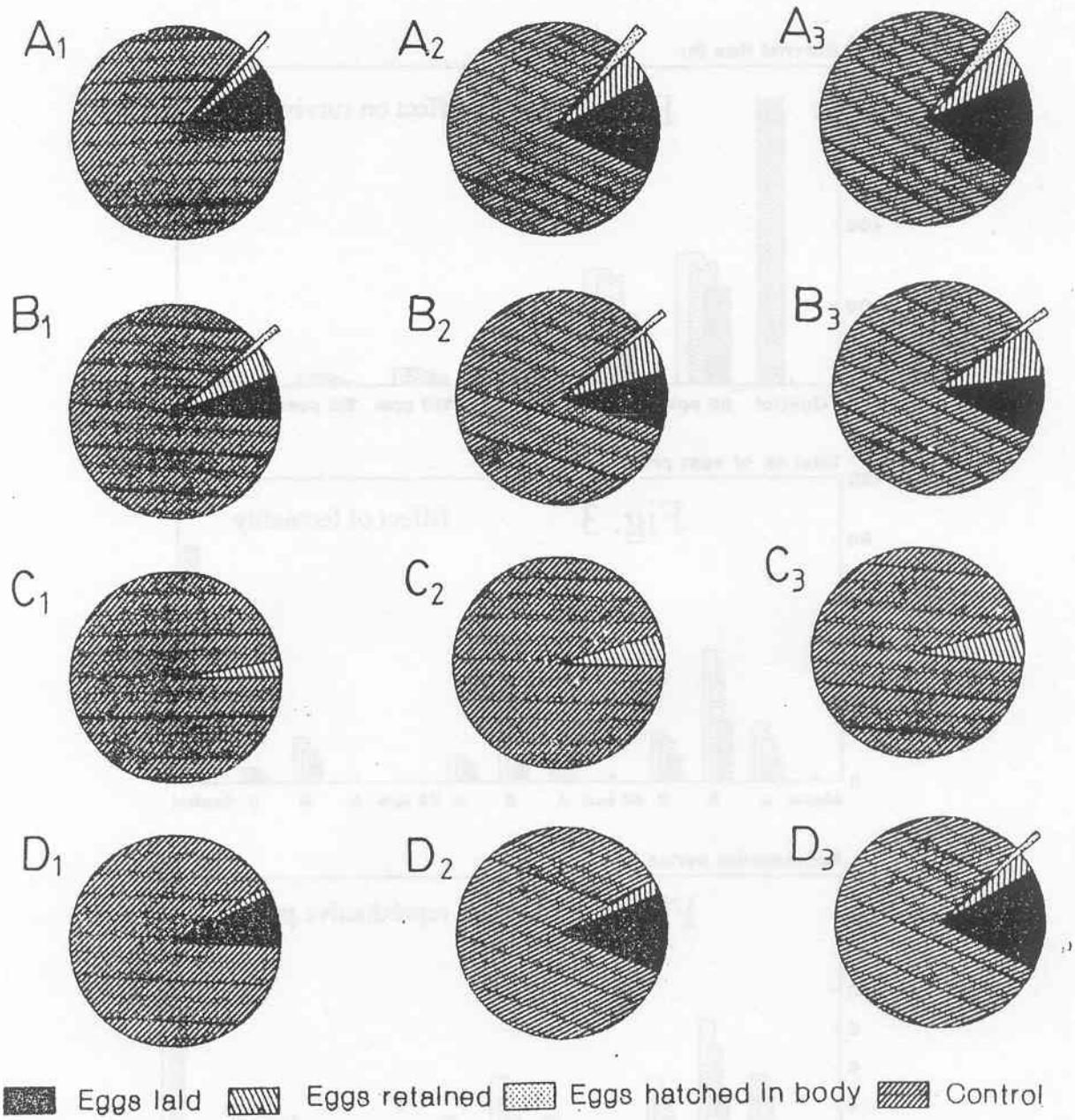

Fig. 5. Nematicidal effect on the intra-uterine egg retention in *Cruznema tripartitum*. A (A1, A2, A3), B (B1, B2, B3), C (C1, C2, C3): Old females in 25, 50 and 75 ppm concentrations of nematicides, respectively. D (D1, D2, D3): Young females in 75 ppm concentration of nematicides.

Survival time (Fig. 2)

In 25 and 50 ppm of nematicides, where the juveniles completed development to adults, the maximum survival time was in phorate, followed by carbofuran and aldicarb. At higher concentrations where development was inhibited, the survival time of the juveniles was drastically reduced but the trend remained the same.

Reproductive period (Fig. 3)

The reproductive period of females developed from treated juveniles or from the young or old females treated with nematicides with 25, 50 and 75 ppm conc. was significantly reduced as compared to control (14-16 days). A gradual decrease in reproductive period length was observed with the increasing conc. of nematicides. Of the nematicides

tested, maximum reduction in reproductive period was seen in aldicarb, and the minimum in phorate whereas the younger females showed a longer reproductive period than the others in any nematicide.

Fecundity and egg-laying (Figs 4 & 5)

The treated juveniles completed development to females which laid eggs in 25 ppm of all nematicides and 50 ppm of carbofuran and phorate. In 50 ppm of aldicarb, egg laying was inhibited. At further higher conc. of nematicides, egg laying did not occur because of incomplete post-embryonic development. Fecundity/egg laying potential was also adversely affected and reduced to one-third or less in the adults developed from treated juveniles as compared to control (70-80 eggs/ female). However, among the treated individuals maximum number of eggs were laid in phorate followed by carbofuran and aldicarb.

In other experiments, the fecundity was higher in young females as compared to the older ones in 25 ppm of nematicides, though it was nearly half of the control in the former. The number of laid eggs gradually declined with the increasing conc. of nematicides and egg laying was completely inhibited in conc. higher than 75 ppm. The older females, however, failed to lay eggs even at 75 ppm concentration.

Under normal conditions (control), the gravid females showed a high tendency of egg retention (60-80% of total eggs produced) subsequently leading to matricidal hatching. In the treated juveniles developing into adults, no intra-uterine development was observed and the retained eggs, 5-10% of the total eggs laid, were always nonviable. The younger females laid eggs in 25 and 50 ppm of all nematicides though intra-uterine retention of eggs (35-75% of the total eggs laid) was observed in older females at the same concentration. In such females 30-50% and 10-20% of the retained eggs showed matricidal hatching in 25 and 50 ppm conc. of nematicides, respectively. The younger females retention of 15-20% eggs was observed first time at 75 ppm conc. though hatching rate was quite low i.e. 10-15%. At the same concentration, retention was 100% in older females though hatching did not occur at all and only 20% of such eggs cleaved up to 4-celled stage and turned nonviable later.

The relative mild nature of phorate in affecting the different developmental activities confirms the assumption that higher dosage levels are required for nematicidal toxicity (Johnson & Feldmesser, 1987). Furthermore, the conclusion of Bunt (1987) regarding the extreme effectiveness of aldicarb in terms of mortality was again supported by the present results which showed that the same conc. of aldicarb proved to be more toxic than others in inhibiting the post-embryonic processes.

The application of nematicides caused delay in moulting and egg laying, a condition also reported in *Caenorhabditis elegans* under the influence of mebendazole compounds (Spence *et al.*, 1982). Studies conducted on the role of carbamates and organophosphates on plant parasitic nematodes have revealed the inhibition of acetylcholinesterase activity (Pree *et al.*, 1987; 1990). Keeping this fact in view, in the present study the delay/inhibition in moulting and egg laying was probably due to impairment of the synaptic processes. Such impairment would have resulted in defective orientation of nematodes thus affecting the feeding ability to a greater extent which would have altered indirectly the growth of different body systems including gonads. Reproductive activity in *C. elegans* due to anthelminthic compounds was reported to be impaired due to muscular dysfunction causing inactivation of gonadal sheath cells which transfer germ cells along the gonadal tube (Bernt *et al.*, 1998). The intra-uterine retention of eggs in the treated females in the present study may be correlated with muscular dysfunction and impaired nervous system which would have affected ageing (older) females the most. In contrast, the adults developed from treated juveniles showed comparatively lesser percentage of retained eggs perhaps due to acclimatisation to the nematicides. The decline in the fecundity rate may be the consequence of blockage in DNA synthesis and thus the cell division (Wright, 1981) resulting in incomplete development of oocytes which were much prone to the toxic effects in the absence of a protective shell barrier.

In plant parasitic nematodes, 5-10 ppm conc. of nematicides seems to be strong enough to cause inhibition of post-embryonic processes (McLeod and Khair, 1975) in contrast to the nematodes under study which were able to complete post-embryonic development at a higher conc. (25-50 ppm) of nematicides. This may be due to the fact that *C. partitum*, which has a short life cycle, received nematicidal exposure for a lesser time than the plant parasitic nematodes which otherwise would require a longer time exposure to complete their development.

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