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Mass Production and Prey Species of *Mylonchulus sigmaturus* (Nematoda: Mylonchulidae) in the Laboratory

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Abstract: The development of mononchid nematodes as biological control agents of phytoparasitic nematodes requires the ability to culture large numbers of these predators. This investigation focused on the propagation of the widely distributed *Mylonchulus sigmaturus*. For mass production, five adults of *Mylonchulus* with 100 prey nematodes (*e.g. Neopsilenchus magnidens, Aphelenchus avenae*, *Helicotylenchus pseudorobustus* and *Ditylenchus* sp.) as a food sources were added to each culture plate containing Soil Extract Agar (SEA) medium. The number of *M. sigmaturus* was recorded 56, 60 and 65 days from the nematode inoculation. The first juveniles were observed on the 56th day, the juvenile number increased to 10 on the 60th day and to 15 on the 65th day. Furthermore, a thin layer of water was needed for activation of the feeding and reproduction of *M. sigmaturus* in the medium. Prey suitability for *M. sigmaturus* was investigated in SEA medium with *A. avenae, H. pseudorobustus*, *Labronema vulvapapillatum,* and larvae of *Meloidogyne javanica* as prey. The results during 21 days of incubation indicated that *M. javanica* and *A. avenae* were excellent sources of prey, whereas the two other species were not consumed.

Keywords: Behaviour, culture, feeding, mononch, media, predation, SEA

Introduction

The majority of predatory nematodes belong to four major taxonomic groups of nematodes including Mononchida, Dorylaimida, Diplogasteroidea, and Aphelenchoidea. Among these, the mononch nematodes are notably important because at their preadult and adult stages they are exclusively predacious; consequently, they have been described as "mini tigers" (Ahmad, Jairajpuri, 2010). Mononchs are frequent inhabitants of the rhizosphere in different kinds of soils, where these predators have significant roles in the food-web ecology (Moens *et al*. 2000). Within the mononchid nematodes, the genus *Mylonchulus* is the most frequent genus found in the soil (Ahmad, Jairajpuri 2010).

The potential of using predatory nematodes for biocontrol of plant-parasitic nematodes in the soil was first suggested by Cobb (1917). Obviously, the use of mononchs as biocontrol agents necessitates the ability to obtain large numbers of these predators. Consequently, several scientists investigated the mass rearing of various mononchid species (Steiner, Heinly 1922, Nelmes 1974, Grootaert, Maertens 1976, Salinas, Kotcon 2005). In the case of *Clarkus papillatus*, Salinas, Kotcon (2005) discovered that mass production *in vitro* required suitable temperature and moisture, as well as prey availability. Different species of nematodes can serve as prey for mononchids (Khan, Kim 2006). For example, *Mylonchulus sigmaturus* can feed on *Heterodera schachtii*, *Meloidogyne javanica*, *Subanguina radicicola*, *Radopholus similis,* and *Tylenchulus semipenetrans* (Thorne 1927, Cassidy 1931, Cohn, Mordechai 1974).

The objectives of this study were: 1) to investigate the mass production of a *M. sigmaturus* population from Iran in the laboratory; and 2) to examine the prey species of *M. sigmaturus* by using different nematode species to determine which is preferred as prey.

Material and Methods

Nematode materials: In this investigation, an artificial environment Soil Extract Agar (SEA medium) (according to Salinas, Kotcon 2005) was prepared to maintain *M. sigmaturus* for the mass production and prey species studies. The specimens of *Mylonchulus* were hand-picked from the soil samples taken from the rhizosphere of wheat plants (*Triticum aestivum*) at the wheat farm of the Shahid Bahonar University of Kerman. SEA medium of 1.5% was prepared and maintained in 10 mm diameter plastic petri dishes. For the mass production investigations, five *M. sigmaturus* adults were transferred to each plate, followed by the addition of approximately 100 adult prey nematodes (*e.g. Neopsilenchus magnidens*, *Aphelenchus avenae*, *Helicotylenchus pseudorobustus,* and *Ditylenchus* sp.; all isolated from the rizosphere of alfalafa except the last species which was isolated from the rizosphere of *Morus alba*) as food sources. The preys were added to the medium one by one in a thin layer of water. All steps were done under sterile conditions. The experiment was performed in a split plot with CRD design, with five replicates. Distilled water (a thin layer of 1-2 mm) was added to each plate. No males were observed at any stage of the experiment. The plates were maintained under 25◦C in darkness. The number of *M. sigmaturus* was then recorded three times (56, 60 and 65 days after the nematode inoculation). For studying the prey species, one adult *M. sigmaturus* was added to each plate, followed by 10 prey specimens, which included *A. avenae, H. pseudorobustus*, *Labronema vulvapapillatum,* or larvae of *Meloidogyne javanica*. The experiment was performed in a split design with CRD, in four replicates. To maintain humidity and activity of the predatory nematodes, a thin layer of water was poured on the surface of the medium and the plates were kept at 25◦C. Data on the number of prey of the *Mylonchulus* and the number of *Mylonchulus* propagated on the medium were analysed using SAS software. The means were compared using Duncan's multiple range test.

Results

Mass production: The number of adult *M. sigmaturus* on the culture plates increased significantly over time (d.f. = 2, F =367, P < 0.0001; Table 1). The number of *Mylonchulus* increased significantly after 56, 60 and 65 days from the inoculation of the nematodes to each plate (P≤0.01; Fig. 1). The first juveniles were observed after 56 days, their number increased to 10 after 60 days, and to 15 after 65 days.

Table 1. Anova of production of *Mylonchulus sigmaturus* in SEA 1.5%

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	1	249.4	249.4	4489.0	<.0001
REP(TREAT)	2	0.1	0.05	1.0	0.4
TIME	2	40.8	20.4	367.0	<.0001
REP*TIME	4	0.2	0.05	1.0	0.5
TREAT*TIME	2	120.8	60.4	1087.0	<.0001

Table 2. Anova of different preys and time in predatory behaviour. Coefficient of Variation (CV) = 3.466926

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	489.6	163.2	1780.6	<.0001
REP(TREAT)	9	10.7	1.2	12.9	<.0001
TIME	10	90.4	9.03	98.6	<.0001
REP*TIME	30	2.1	0.07	0.8	0.8
TREAT*TIME	30	122.2	4.07	44.4	<.0001

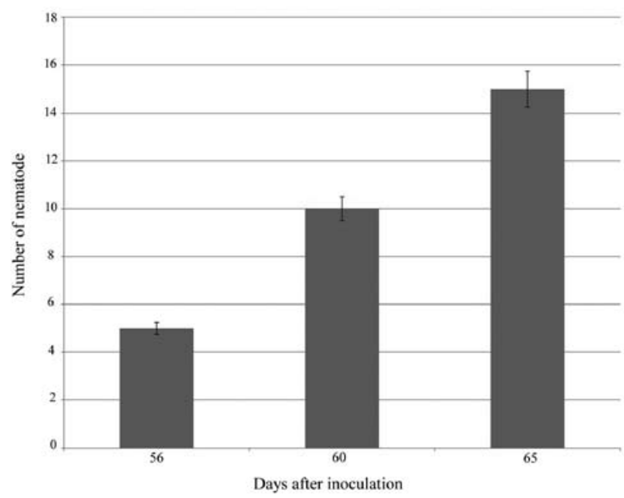

Fig. 1. Production of *Mylonchulus sigmaturus* in 1.5% Soil Extract Agar

Prey species: The study of the predatory behaviour demonstrated that the prey number in the culture plates decreased significantly over time in the presence of *M. sigmaturus* (d.f. = 10, F =98.6, P < 0.0001; Table 2). The experiment also revealed that L2 of *M. javanica* was a suitable food source for *M. sigmaturus* (Fig. 2). Of the four prey species used in this experiment (*L. vulvapapillatum*, *H. pseudorobustus*, *A. avenae,* and L2 of *M. javanica*), L2 of *M. javanica* and *A. avenae* were consumed by the predatory nematode significantly (P≤0.01). From days 3-21, the lower number of L2 of *M. javanica* compared to other species indicates that this species was more suitable as prey for *M. sigmaturus* than the

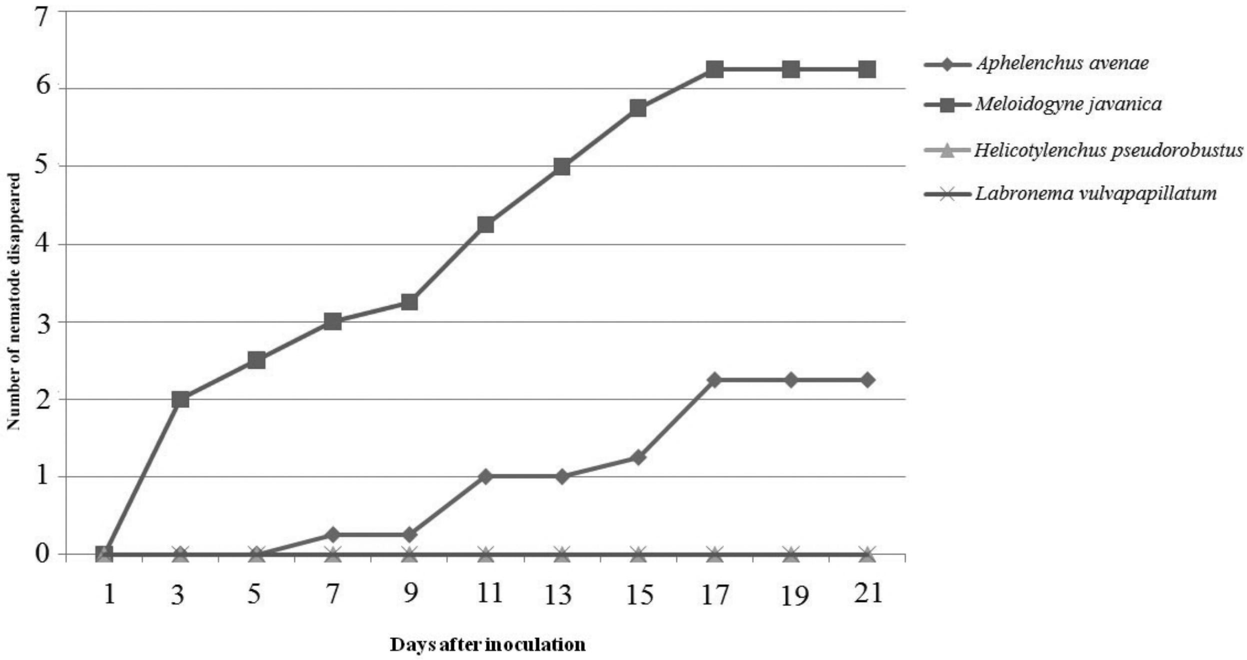

Fig. 2. Prey species of *Mylonchulus sigmaturus* in total plates

other potential food sources used in this study.

Discussion

Mononchid nematodes reproduce on nematode prey in SEA medium (Salinas, Kotcon 2005). This medium has been used as a substrate for mononchs because it is widely available and easily handled. In the present study, we successfully maintained *M. sigmaturus*, as the number of nematodes increased during time. We also found that a film of water is necessary for establishment of the nematode on the medium. Similarly, Salinas, Kotcon (2005) reported that a water layer on the agar is essential for maintaining *C. papillatus*. Although Nelmes (1974) noted that *Prionchulus punctatus* can stay on the medium without a layer of water, Cassidy (1931) indicated that a water layer is needed for maintaining *Iotonchus brachylaimus* and used a mixture of plant-parasitic nematodes as prey for this species. The cannibalism observed by Salinas, Kotcon (2005) was not ascertained in our study. Although we did not attempt to sterilize the nematodes before transfer to the plates, any contamination was not observed, in agreement with Salinas, Kotcon (2005). Saur, Arpin (1989) revealed that mononchs can feed on soil clay particles, bacteria and other microorganisms. Yeates (1987) mentioned that *Clarkus propapillatus* can be maintained in a medium with bacteria for 8 months.

The nematodes belonging to the order Mononchida are predacious in nature (Jairajpuri, Azmi 1978, Jairajpuri, Khan 1982). Our results demonstrated that *M. sigmaturus* can feed on *Meloidogyne* as suitable prey. Similar reports of mononch predation on *Meloidogyne* were obtained for *C. papillatus* by Cobb (1917) and Steiner, Heinly (1922). Cohn, Mordechai (1974) reported that a large population of *M. sigmaturus* may reduce the population of *T. semipenetrans*. *Prionchulus punctatus* reduced significantly the population density of *Globodera rostochiensis* and *M. incognita* in pot experiments (Small 1979). Azmi (1983) revealed that *Mulveyellus monhystera* can decrease the population density of *Helicotylenchus dihystera*. Rama, Dasgupta (1998) noted that in the soil of Mandarin orange orchards with *Iotonchus tenuicaudatus*, the plant parasitic nematodes *T. semipenetrans* and *H. dihystera* were observed in low population.

With respect of the feeding ability of *M. sigmaturus*, the larvae of *M. javanica* and *A. avenae* were mostly preferred; *H. pseudorobustus* and *L. vulvapapillatum* were not successfully preyed upon. It also became clear that consumption of the larvae of *Meloidogyne* was faster than of *Aphelenchus*. This difference could be due to the smaller size of the *Meloidogyne* larvae, which result in easier feeding on them by the predatory nematodes. Similarly, Jairajpuri, Azmi (1978) demonstrated that *Mylonchulus dentatus* prefers *Meloidogyne* and *A. avenae* but not *Hoplolaimus indicus* as prey. According to some scientists (*e.g.* Small, Grootaert 1983, Bilgrami, Jairajpuri 1989), the thick cuticle (*Hoplolaimus*), coarse body annulations (*Hemicriconemoides*), gelatinous matrix (*Meloidogyne* egg mass), toxic body secretions (*Helicotylenchus*), and the rapid undulatory body movements (*Rhabditis*) prevented the mononchid nematodes from effective feeding on the mentioned taxa.

The Mononchida offer potential as useful biological control agents of plant-parasitic nematodes (Cassidy 1931, Christie 1960). Although their predatory feeding on *Meloidogyne* spp. and other major plant-parasitic nematodes is a successful ecological strategy, further investigations of mononch predation in field situations are needed before they can be used as biocontrol agents.

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