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RESEARCH ARTICLE

Oribatid mites as potential predators of the root knot nematode, *Meloidogyne incognita*

Nithinya RAMAKRISHNAN [1,3], **Ramani NERAVATHU** [2]

[1] *Department of Zoology, Sree Narayana College, Kannur, Kerala, India*
[2] *Department of Zoology, University of Calicut, Kerala, India*
[3] Corresponding author: rnithinyasnc@gmail.com

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ABSTRACT: Oribatid mites, which represent the most dominant group among the soil mites, have considerable diversity in their feeding habits. Nematodes quite often serve as the most favoured prey choice for oribatids, a situation which could be better exploited in biocontrol strategies. Hence the present study was carried out to explore the predatory habits of selected species of oribatid mites on a notorious nematode pest, namely the root knot nematode, *Meloidogyne incognitia*, under laboratory conditions. The study was commenced by collecting soil samples from various localities of Kannur and Malappuram districts of Kerala, India in the period March, 2014 to February, 2015. Among the various species of oribatid mites extracted, three *Scheloribates* species, *viz. Scheloribates* (*Scheloribates*) *praeincisus* (Berlese, 1910), *Scheloribates fimbriatus africanus* Wallwork, 1964 and *Scheloribates* (*Scheloribates*) *latoincisus* Hammer (1973), were selected for studies of their predatory potential on the second stage juveniles (J2 juveniles) of *M. incognita.* During feeding experiments each of the three mite species was offered 20 J2 juveniles of *M. incognita* in petri dishes containing 2% sterilized water agar. Observations were made on the feeding behavior of the species, including rate of consumption, for comparative evaluation. The per day percentage consumption of J2 juveniles by *S.* (*S.*) *praeincisus*, *S. fimbriatus africanus* and *S.* (*S.*) *latoincisus* were 57.00 ± 7.3598, 60.00 ± 6.3738 and 57.00±5.9675, respectively. Statistical analysis following one way ANOVA and Turkey's HSD Post Hoc Test, showed no significant difference in the consumption rates among the species ($P = 0.452 \geq 0.05$). It is concluded that the three *Scheloribates* spp. have equivalent potential to suppress root knot nematode populations.

Keywords: Oribatid mites, predation, root knot nematode, *Scheloribates*, biocontrol.

INTRODUCTION

Soil is a complex and dynamic ecosystem that contains diverse floral and faunal communities. Faunal components are dominated by arthropods, followed by nematodes. Among the soil arthropods, mites especially oribatid mites, outnumber other mesofaunal elements (Petersen and Luxton, 1982). Oribatid mites have a critical role in the environment, including enhancement of porosity, aeration, mixing of soil and its many microbes, biodegradation of organic residues, nutrient cycling of soil, bioindication of soil health status and pollution, and biocontrol of pests, parasites and weeds (Krantz and Walter, 2009). The elimination of mites was reported to result in a 40% reduction in decomposition, suggesting that mites would affect the decomposition of buried litter by regulating the population size of bacterial grazers and cephalobid nematodes (Gan et al., 2014).

Plant parasitic nematodes feed on all parts of plants. In different ways with their specialized feeding apparatus called the stylet. Root knot nematodes of the genus *Meloidogyne* are the most notorious plant parasites, infesting about 2,000 plant species, including food crops, vegetables, fruit and ornamental plants in tropical, subtropical and temperate regions, causing approximately 5% loss of global crops (Sasser and Carter, 1985). For those *Meloidogyne* spp. that have host ranges that are large (total number of hosts) and broad (large number of plant families with species susceptible to the nematode), there are quite distinct differences in their overlapping host ranges (Perry et al., 2009). Infestation results in symptoms like chlorosis, distortion of root structure and gall formation, and reduced growth and yield. Severe infestation leads to complete deformation of the root system, hindering nutrient and water uptake, which ultimately leads to death of the plant. Populations of plant parasitic nematodes are kept in check by soil arthropods like soil mites, in undisturbed and agricultural ecosystems (Gulati, 2012).

During embryonic development species of *Meloidogyne* passes through four juvenile stages (J1-J4) before final transformation into adult. The first stage juvenile molts within the egg, embedded in the root of the host plant and hatches as a second stage juvenile. After hatching, the motile infective J2juvenile stage migrates through the soil and may re-enter the same host plant or enter the root of another suitable host plant (Eisenback and Triantaphyllou, 1991).

An early study on the biocontrol of nematodes was made by Linford and Oliveira (1938), they collected many biotas that were antagonistic to the root-knot nematode, *M. marioni* in Hawaiian soils. Their studies presumed six mites of undetermined taxonomic status to restrict nematodes. Since then, many nematophagous mites obtained from several acarine cohorts have been documented. Apart from the disciplinary disjunction between soil acarology and nematology, incorrect assumptions have de-

layed understanding of the strong interaction between mites and nematodes. It was generally believed that mites and nematodes rarely come in close proximity which would inhibit the evolution of complex relationships like predation to develop between them. Nematodes are slender and soft bodied, and they can enter very small soil-pore spaces unavailable to mostly broader and less flexible mites. However, many nematophagous mites are elongate, flexible, and capable of extruding their chelicerae a considerable distance into small pores (Walter and Proctor, 2013).

Nematophagy has been reported in various groups of mites like the Mesostigmata, Astigmata and Cryptostigmata (Rocket et al., 1966; Rocket, 1980; Koehler, 1997; Bilgrami, 1993; Al Rehiayani and Fouly, 2005; Olivera et al., 2007) but still represents a very poorly explored area of research. Hence, the present study was undertaken to evaluate the feeding and biocontrol potential of 3 species of mites belonging to the suborder Oribatida on one of the most notorious root knot nematodes, *M. incognita*, which induces root galls in the tomato (*Solanum lycospersicum)*.

MATERIAL AND METHODS

Methods adopted for obtaining root knot nematode

Twenty-five samples of nematode infected tomato roots were collected from each of two localities, namely Kannur and Malappuram districts of Kerala, India in the period March, 2014 to February, 2015. Roots were placed in polythene bags, sealed, labelled and then brought to for examination to the Soil Biology Laboratory, Department of Zoology, University of Calicut, Kerala, India. Root knots were gently teased apart by using a needle to facilitate the collection of males and females. Females were used for identification based on the perineal pattern on the cuticle. J2 juveniles of *Meloidogyne incognita* were extracted with a Sieve-Petri dish setup, which consisted of a plastic sieve placed between two Petri dishes, with the lower Petri dish filled with water. The infested root samples were retained on the plastic sieve and left completely immersed in water overnight. J2 juveniles that hatched out into the water in the lower Petri dish were collected and used for the experiment.

Collection and culturing mites

Twenty soil samples were collected from two districts, Kannur and Malappuram, Kerala, India from March 2014 to February 2015. The samples were collected in polythene bags. Oribatid mites were extracted by using an open brass funnel apparatus (Haq and Ramani, 2002). Live specimens were isolated and then cultured on leaf, wood, moss and litter in cylindrical plastic vials (3.8 cm diameter, 4.5 cm height). The base of the culture vessel was filled to 2 cm depth with a solid mixture of plaster of Paris and charcoal (4:1 ratio). The mites were cultured at room temperature (31^0C, RH 74%).

Processing of mites for taxonomic studies

The mites for taxonomic studies were preserved in 70% alcohol and were dehydrated by passing through an alcohol series (90% and then absolute alcohol). When the mites were completely dehydrated, they were transferred to a clearing medium prepared by mixing lactic acid and alcohol (1:1 ratio). Based on sclerotization, the mites required a few days to several weeks or months for clearing. The cleared specimens were temporarily mounted in glycerine. Permanent slide mounts were prepared in Hoyer's medium. Glass bristles (0.5 mm thick) were added to mounting medium in order to prevent the specimen from being crushed under the weight of coverslip. Mounted specimens were identified by using the following keys (Balogh, 1965, 1972; Balogh and Mahunka, 1983; Balogh and Balogh, 1988, 1990, 2002; Subias, 2012 and edition updated in 2014).

Experimental design of preliminary feeding trials

Screening of mites with predatory potential was carried out in culture cells with a base made from a plaster of Paris – Charcoal mixture (4:1). Twenty J2 stages of *M. incognita* in 0.5 ml tap water were transferred separately to different culture cells. The water was soon absorbed by the Plaster of Paris –Charcoal mixture, leaving the nematodes exposed at the bottom of the culture cell. Two adult female mites of selected species, starved for 24hrs were then released into the cell. The feeding behavior displayed by the tested species of mites was checked under a Stereozoom microscope (Macro Vis, USA - Model No.MVNSZ-405). Behaviour of the mites was noted every hour for 6 hours, after which the number of the remaining J2juveniles was counted. Based on the results of this preliminary feeding study, mites with predatory potential were identified and considered for detailed experiments.

Conduct of feeding experiments

Experiments were conducted in Petri dishes (40 mm diameter and 10 mm deep) containing a 4 mm deep layer of 2% sterilized water agar at room temperature 31^oC-32^oC and RH 74-82%. Each treatment in every experiment had 4 replicates. The experiment was commenced with the starving for 24 hours of a single female mite (each of the three species) selected during the preliminary studies (day 1 of experiment).

Twenty J2 juveniles of *M. incognita* in 0.5 ml tap water were then added to the central part of the Petri dish. After the water drop had been completely absorbed by the agar, the starved female mite was placed in the Petri dish. Each Petri dish was covered with a piece of parafilm (day 2 of experiment).

The feeding response of adult mite on the nematodes was observed every two hours under a stereozoom microscope. After 24 hrs, the mite was removed and the Petri dishes were rinsed with distilled water three times to collect the remaining nematodes in a counting chamber and the number of nematodes was counted under stereozoom microscope (Macro Vis, USA - Model No.MVNSZ-405). The feeding experiments were carried out with mites that were 35 days old (all the *Scheloribates* spp. used here took a maximum of 33 days for development

from egg to adult stage at room temperature 31°C -32°C and RH 74-82%).

The mites were then starved again for 24 hrs (day 3 of experiment) and then provided with nematodes on the fourth day. The experiment was repeated until the 10th day, with each day of nematode inoculation followed by a day of starvation.

Multiple-food choice preference test

The multiple-food choice test, was carried out in cylindrical plastic culture vessels (3.8 cm diameter and 4.5 cm height) and having a base of plaster of Paris - Charcoal mixture (4:1). Adult female mite (each of the three selected mite species) was starved for 48 hrs and placed precisely at the central portion, on the base of plastic vessel. Five live specimens each of *M. incognita* female, male and J2 juvenile were placed at equal distance from each other, at a distance of 1 cm from the mite at the centre. The experiment was carried out in 5 replicates. Feeding behaviour was observed for 1 hr and observations on feeding preference were noted.

Statistical Analysis

The experimental results are presented as mean ± SD. Statistical evaluation of data was done with one way ANOVA and Turkey's HSD Post Hoc Test. Results were considered statistically significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

The present study explored the predatory potential of three species of *Scheloribates* – *S.* (*S*). *praeincisus*, *S. fimbriatus africanus* and *S.* (*S.*) *latoincisus* on J2 juveniles of *M. incognita* (Figs 1A-C). Among the 3 selected *Scheloribates* species selected for study, the highest predation was exhibited by adults of *S. fimbriatus africanus* with a per day average feeding rate of 12.00±1.2748 (60 ± 6.3738%); followed by S. (*S.*) *latoincisus* 11.4 ±1.1937 (57 ±5.9675%) and *S.* (*S.*) *praeincisus*, 10.6±2.3822 (57.500±7.3598) (Fig. 2). The statistical analysis of data by one way ANOVA (with Turkey's HSD Post Hoc Test) revealed no significant difference in the per day feeding rate between the *Scheloribates* species on J2 juveniles of *M. incognita* ($p \geq 0.05$). Therefore, the three *Scheloribates* species appear to have equivalent potential to suppress root knot nematode populations.

In the present study, the per day average consumption rate of the three *Scheloribates* species (*S. fimbriatus africanus*, *S.* (*S.*) *latoincisus* and *S.* (*S.*) *praeincisus*), on J2 juveniles of *M. incognita* was 11.3. This value is less than the mean per day consumption rate (18.3± 0.8 per day) of J2 juveniles of *M. javanica* by adults of *Pergalumna* sp., an oribatid mite (Oliveira et al., 2007). This difference in feeding rate may be attributable to differences in the prey or predator species, or both, and the experimental conditions.

The current study revealed the feeding behavior of *Scheloribates* (Fig. 1D). The mites generally devoured the entire bodies of J2 juveniles but when disturbed the prey were left without completing consumption. Around 60-80 sec was required for complete consumption. While feeding, the mites exhibited up and down as well as side to side movements of the anterior part of the body. No regional specificity to the body of the prey was observed among the *Scheloribates* species; the mites actively moved around the experimental area and initiated feeding at the site of first contact with the prey body.

None of the three selected mites studied exhibited a preference for live adult females of *M. incognita* but fed on adult males of *M. incognita* offered during feeding trials (Fig. 3). The oribatid mites probably did not feed on *M. incognita* females because of the large, pear-shaped body (671 x 600 µm) of females. All the mites studied preferred the J2 stages of the nematode when compared with adults ($p \geq 0.05$). A similar preference for the juveniles compared to females of *Meloidogyne* was reported for predation by oribatid mites (Muraoka and Ishibashi, 1976).

Under natural conditions it would be rare for soil mites to directly encounter adults of *M. incognita* since soil mites are usually restricted to rhizhosphere, while *M. incognita* adults are found within the roots of plants. In this scenario, the biocontrol of *M. incognita* by oribatid mites like *Scheloribates* would appear unlikely, but the J2 juveniles of *M. incognita* hatch into the soil before infesting the root of the same plant or a neighboring host plant.

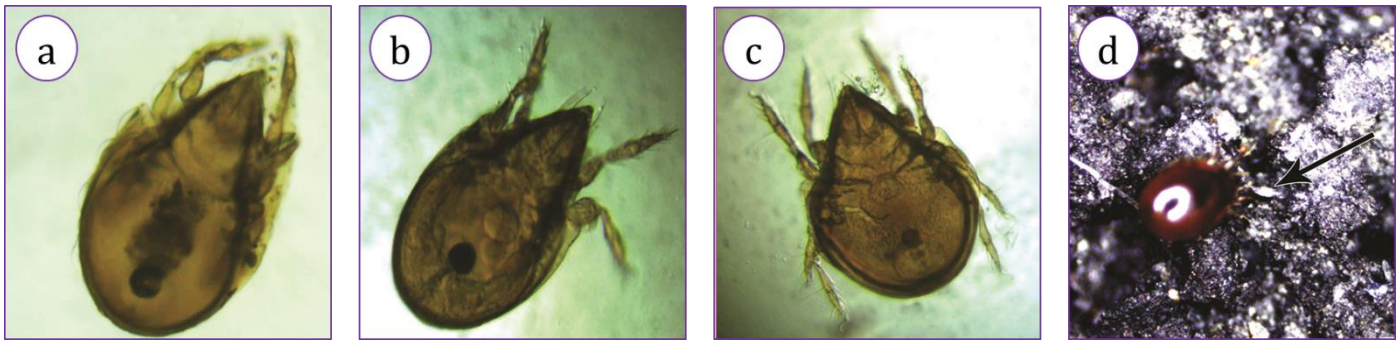

Figure 1. A) *Scheloribates* (*Scheloribates*) *praeincisus* (Berlese, 1910). B) *Scheloribates fimbriatus africanus* Wallwork, 1964. C) *Scheloribates* (*Scheloribates*) *latoincisus* Hammer, 1973. D) *Scheloribates* mite feeding on J2 juvenile of *M. incognita*.

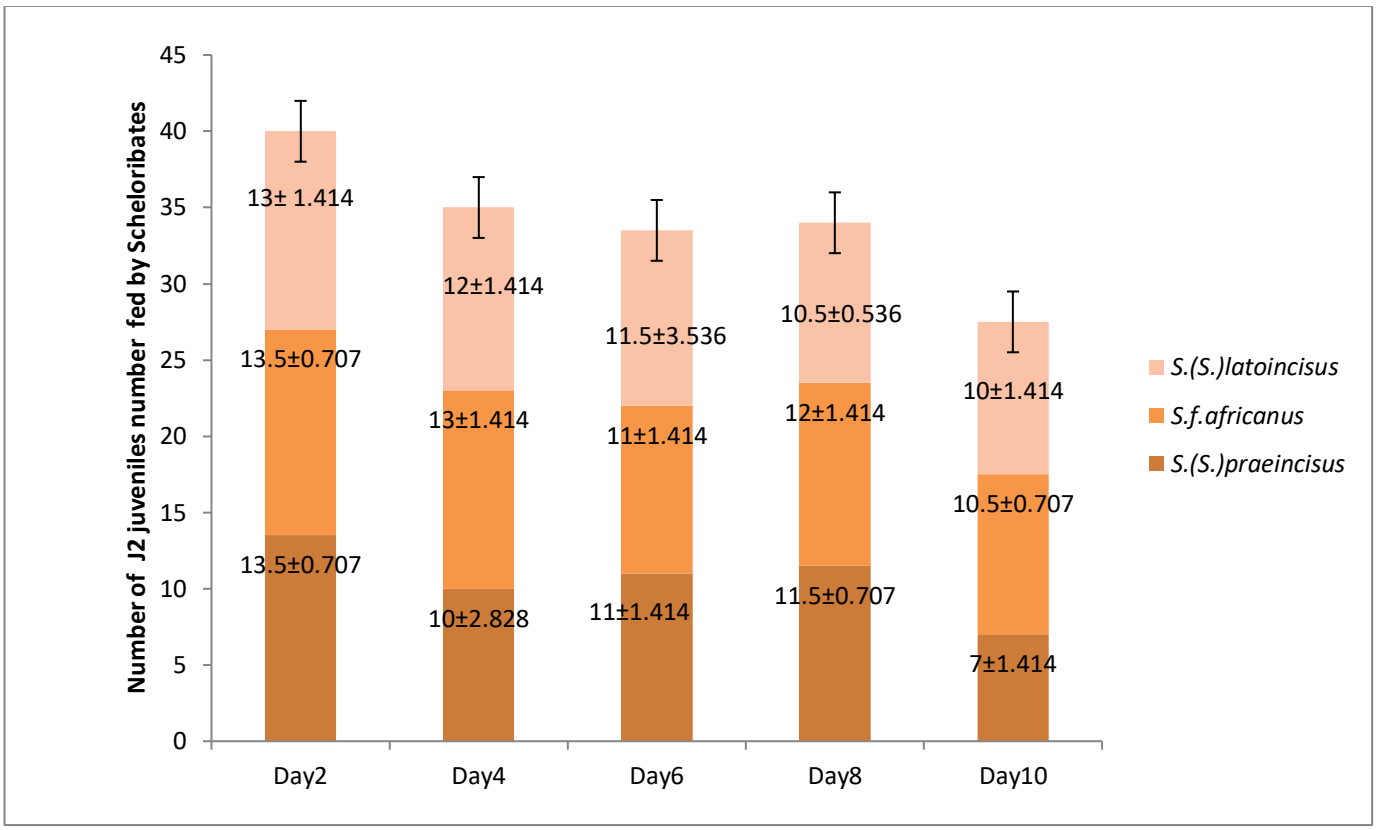

Figure 2. Mean rate of predation of J2's of *M. incognita* by *Scheloribates* species.

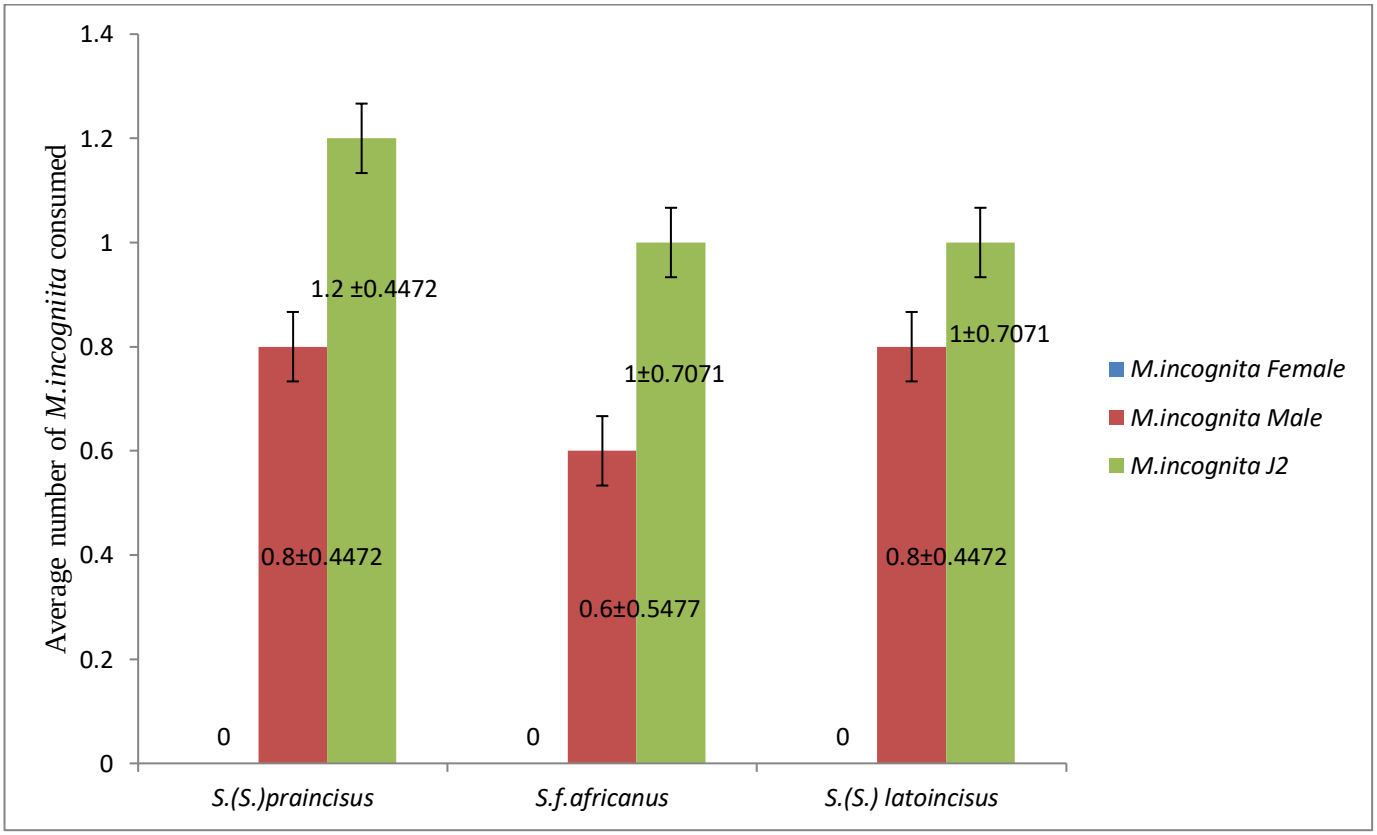

Figure 3. Feeding preferences of *Scheloribates* species on *Meloidogyne incognita*.

The *Scheloribates* mites in the soil could effectively predate on these juvenile nematodes. The predation by oribatid mites on J2 juveniles of *M. incognita* could not only decrease the nematode load in the soil but also reduce the spread to the next host. Moreover, the role of oribatid mites in biodegradation would enhance soil fertility (Haq, 2016). The results of the present study helped to establish three oribatid species as prospective biocontrol agents of *M. incognita*. More laboratory studies and field oriented studies are warranted to explore the potential of predatory mites for inclusion in integrated pest management (IPM) strategies. In addition, the potential of oribatid mites could be exploited not only for the reduction of numbers and spread of root knot nematodes but also for the enhancement of soil fertility and crop production.

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