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PREDATORY BEHAVIOUR OF TWO NEMATOPHAGOUS MITES, *TYROPHAGUS PUTRESCENTIAE* AND *HYPOASPIS CALCUTTAENSIS*, ON ROOT-KNOT NEMATODE, *MELOIDOGYNE JAVANICA*

by
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Summary. The feeding and predatory behaviour of two nematophagous mites viz., *Tyrophagus putrescentiae* and *Hypoaspis calcuttaensis* was studied *in vitro* on *Meloidogyne javanica*. *T. putrescentiae* consumed any nematode prey encountered while wandering; it fed on one nematode for sometime, left it injured and started feeding on the other. *H. calcuttaensis* actively searched for its prey and predated continuously; it devoured the nematode *in toto*. The mouth parts are suggestive of this differential feeding behaviour of the two mite species. Both the mite species fed voraciously on the vermiform stages and free eggs of nematodes. Maximum number of nematodes were consumed within the first 24 hrs. *T. putrescentiae* consumed eggs within eggmasses also, while *H. calcuttaensis* failed to do so. Both the mite species devoured dead nematodes more efficiently than live ones. All the active developmental stages (larvae, nymphs and adults) of mites were found nematophagous, however, the adults of both the mite species and nymphs of *H. calcuttaensis* were better predators compared to rest of the developmental stages. Larvae of *H. calcuttaensis* consumed least number of nematodes. Temperature influenced the rate of predation, maximum predation occurred at 25 °C.

The trophic behaviour of soil acarines is not well understood due to their cryptic habitat and difficulty in culturing them *in vitro*. There are a lot of reports, mostly based on causal observations, indicating nematophagy as a common phenomenon among soil acarines. The nematophagous mites have been reported from diverse groups but most of the predacious forms belong to order Mesostigmata (Walter, 1988a). Nematophagous mites have been found to be widespread in agricultural soils. Of the 15 genera of mesostigmatid mites prevalent in the rhizosphere of different crops, 12 were observed to feed and survive on nematodes during *in vitro* studies (Walia, 1992).

There is a lack of information on the predatory activity of astigmatid mites on nematodes. Among these mites, *Tyrophagus putrescentiae* (Schrank) (Astigmata: Acaridae) has been reported preying on plant and soil nematodes (Walter *et al.*, 1986; Bilgrami, 1994). *In vitro* studies were conducted to compare the predacious behaviour of *T. putrescentiae* with that of *Hypoaspis calcuttaensis* Bhattacharya (Mesostigmata: Laelapidae) using as a prey the root-knot nematode *Meloidogyne javanica* (Treub.) Chitw. and *Heterodera avenae* Woll. The results of these studies are reported in this paper.

Materials and methods

T. putrescentiae and *H. calcuttaensis* were cultured on wheat flour: yeast (4:1) and *Fusarium solani* (Sacc.) Mart. emended Synd. *et* Hans

+ *Aphelenchoides composticola* Franklin, respectively in the laboratory. The following six experiments were conducted to test the predatory activity of the two mite species, *in vitro*:

a) rate of predation on vermiform nematodes (second-stage juveniles of *M. javanica*);

b) rate of predation on nematode eggs (free eggs of *H. avenae*);

c) predation on the egg masses of *M. javanica*;

d) predation on dead and live nematodes (second-stage juveniles of *M. javanica*);

e) predation potential of different active stages of mites (on second-stage juveniles of *M. javanica*);

f) influence of temperature on predation by mites (on second-stage juveniles of *M. javanica*).

All the experiments were conducted in laboratory using observation blocks made of plaster of Paris: charcoal (9:1) having a central cavity of diam 1 cm, depth 0.5 cm. Approximately 1000 second-stage juveniles of *M. javanica* or *H. avenae* eggs (obtained by crushing cysts in water) were pipetted in the central cavity of the observation blocks in 5 ml of water suspension. Egg masses of *M. javanica* were transferred with a dropper. A copulating pair of *T. putrescentiae* / two adult females of *H. calcuttaensis* were released into the central cavity, the blocks were immediately covered with a glass slide held in position by rubber bands. The blocks were further moistened by touching their underside in water, before transferring them to desiccators containing saturated solution of potassium chloride for *T. putrescentiae* and potassium dihydrogen phosphate for *H. calcuttaensis*.

The experiments were set at 25±1 °C, except in experiment f), for which three different temperatures i.e., 20, 25 and 30 °C were tested. In experiment c), the egg masses of *M. javanica* were killed in hot water (65-70 °C for 5 mins). Similarly, the nematode suspension for experiment d) was heated to 60-65 °C for 1-2 mins to kill the juveniles. At the end of experiments the contents of central cavity were flushed out with water and counted under stereomicroscope. The observations on the eggs or juveniles were recorded after 24, 48 and 72 hrs in experiments a) and b); 48 hrs in experiment c); 24 hrs in experiments d) and f); and after 6, 12 and 24 hrs in experiment e). In control (without mites), the recovery of nematodes was always a little less than the initial number. The difference was deducted from other treatments also to offset the error involved in this technique.

Results

Predatory behaviour - general observations

The following observations on the feeding behaviour of the two species were recorded during experiments. *T. putrescentiae* did not actively search for nematodes, but fed only on some nematodes encountered while wandering. When feeding, it used its chelicerae, partially fed on one nematode and moved to another. *H. calcuttaensis* devoured the nematodes *in toto*. It first captured the prey in 15-30 sec and rolled it into a ball, holding the nematode between pedipalps and chelicerae. With the continuous movement of chelicerae, the nematode was consumed completely. The whole process from capturing to engulfing the prey lasted about 1 min. Over a 10 min period of scanning, the mite was seen feeding on nematodes continuously. Capturing of nematodes was not a matter of chance but the mite searched and explored the area for nematodes.

Rate of predation

On vermiform nematodes *H. calcuttaensis* consumed 79.9% nematodes within 24 hrs in contrast to 65.4% by *T. putrescentiae*. *H. calcuttaensis* maintained a significantly higher rate of predation up to 48 hrs but by 72 hrs both species had consumed 92.8% of the nematodes (Table I). The maximum number of nematodes (72.6%) were consumed in the first 24 hrs. However, the rate of predation decreased with the length of time for which the mites fed on the nematodes.

Eggs of *H. avenae* do not hatch at 25 °C. *H. calcuttaensis* maintained a higher rate of predation on the eggs too at all the three periods, though the difference was not significant at 24 hrs. By the end fo 72 hrs, *T. putrescentiae* consumed 96.2% while *H. calcuttaensis* devoured 97.4% eggs. Most of the eggs (81.1%) were consumed within the first 24 hrs and the rate of predation decreased thereafter. Comparing the means of period, *H. calcuttaensis* showed better predacious activity than *T. putrescentiae*, the corresponding figures being 91.4 and 89.0%, respectively (Table II).

On nematode egg masses *T. putrescentiae* fed on the dead eggs protected within the gelatinous matrix and consumed 72.7% eggs in 48 hrs which was significantly higher than that of *H. calcuttaensis*. The latter apparently failed to feed on the egg masses and consumed a negligible (3.2%) number of eggs only (Table III). *T. putrescentiae* was observed to penetrate inside the egg masses, while *H. calcuttaensis* was unable to do so and moved on the surface only.

Both the mite species consumed more dead (82.6 and 98.6% by *T. putrescentiae* and *H. calcuttaensis*, respectively) than live nematodes (75.3% and 82.9%, respectively). *H. calcuttaensis* was better predator than *T. putrescentiae* both in terms of dead and live nematode consumption (Table IV).

Predation by different developmental stages of mites

The larvae, nymphs and adults of both the mites fed on nematodes. The adults of both the mites and nymphs of *H. calcuttaensis* consumed a significantly higher number of nematode juveniles than other developmental stages. The larvae and nymphs of *T. putrescentiae* consumed 56.5 and 51.8% of the nematodes, respectively. However, larvae of *H. calcuttaensis* consumed only 31.7% of the nematodes. The rate of predation decreased with time (Table V). The predacious activity of adults and nymphs of *H. calcuttaensis*, larvae and adults of *T. putrescentiae* after 24 hrs; and nymphs of *H. calcuttaensis* and adults of *T. putrescentiae* after 12 hrs were statistically at par.

TABLE I - *Rate of predation of* Tyrophagus putrescentiae *and* Hypoaspis calcuttaensis *on the second-stage juveniles of* Meloidogyne javanica *(mean of 10 replicates).*

Mite species	Per cent juvenile consumption after			Mean
	24 hrs	48 hrs	72 hrs	
T. putrescentiae	65.4 (54.1)	75.8 (60.1)	92.8 (74.9)	77.9 (63.0)
H. calcuttaensis	79.9 (63.7)	86.4 86.4)	92.8 (74.9)	86.3 (69.1)
Mean	72.6 (58.9)	81.1 (64.4)	92.8 (74.9)	

Figures in parentheses are angular transformed values
Initial number of mites = 2 adults
Initial number of nematodes = 1000

C.D: (5%)
Mites = (1.8)
Period = (2.7)
Mites x Period = (3.8)

TABLE II - *Rate of predation of* T. putrescentiae *and* H. calcuttaensis *on the eggs of* Heterodera avenae *(mean of 10 replicates)*

Mite species	Per cent egg consumption after			Mean
	24 hrs	48 hrs	72 hrs	
T. putrescentiae	80.9 (64.1)	90.0 (71.9)	96.2 (78.8)	89.0 (71.6)
H. calcuttaensis	81.4 (64.8)	95.3 (77.8)	97.4 (80.8)	91.4 (74.5)
Mean	81.1 (64.4)	92.3 (74.9)	96.8 (79.8)	

Figures in parentheses are angular transformed values
Initial number of mites = 2 adults
Initial number of nematodes = 1000

C.D. (5%)
Mites = (1.8)
Period = (2.1)
Mites x Period = (3.0)

Influence of temperature on predation

The maximum predation (82/9%) occurred at 25 °C, while feeding at 20 °C (74.6%) and 30 °C (75.2%) were not statistically different. Comparing the two mite species, *H. calcuttaensis* consumed 90.4% nematodes as compared to 64.8% in case of *T. putrescentiae*. However, predacious activity of *T. putrescentiae* was similar at 20 °C (70.4%) and 25 °C (68.1%) but it was significantly less at 30 °C (55.7%), while in case of *H. calcuttaensis*, 25 °C was the best temperature for predation (97.7%) followed by 30 °C (94.7%) and 20 °C (78.8%) (Table VI).

Discussion

Contrasting differences between the feeding activity of *H. calcuttaensis* and *T. putrescentiae* could be correlated with their cheliceral morphology. *T. putrescentiae* which possesses a few teeth on the fixed digit and movable digit seems to be more adapted for cutting and biting – a characteristic feature of fungivorous and granivorous or omnivorous mites (Wolley, 1988). Bilgrami and Tahseen (1992) and Bilgra-

TABLE III - *Predation by* T. putrescentiae *and* H. calcuttaensis *on the eggmasses of* M. javanica *(mean of 10 replicates)*

Mite species	Per cent egg consumption after 48 hrs
T. putrescentiae	72.7 (58.5)
H. calcuttaensis	3.2 (9.5)

Figures in parentheses are angular transformed values
Initial number of mites = 2 adults
Initial number of eggmasses = 2
Mean number of eggs per eggmass = 250

TABLE IV - *Predation by* T. putrescentiae *and* H. calcuttaensis *on the dead and live second-stage juveniles of* M. javanica *(mean of 5 replicates).*

Mite species	Nematodes	Per cent juveniles consumed after 24 hrs
T. putrescentiae	Dead	82.6 (65.3)
	Live	75.3 (60.3)
H. calcuttaensis	Dead	98.6 (84.6)
	Live	82.9 (65.6)
C.D. (5%)		(4.3)

Figures in parentheses are angular transformed values
Initial number of mites = 2 adults
Initial number of nematodes = 1000

mi (1994) reported intermittent nematode feeding by this mite on a number of nematode species. Bilgrami (1994) also observed that small nematodes (second-stage juveniles of plant parasites) were killed more than larger ones (predacious nematodes) by *T. putrescentiae*. *H. calcuttaensis* has a number of small teeth on fixed digit and a few large teeth on movable digit which are suggestive of active predation on nematodes or other worm-like prey. Karg (1983), Walter and Lindquist (1989) and Buryn and Brandl (1992) have suggested that mesostigmatid nematode specialiste have stout cheliceral digits with large number of teeth on fixed digit and movable digit.

Attempts to observe nematode remains in the gut of the mites were unsuccessful. Similar efforts made by others (Walter, 1987, 1988 b) also failed to produce recognizable gut contents.

H. calcuttaensis failed to consume eggs embedded in the egg masses of *M. javanica* while *T. putrescentiae* could do so by penetrating inside the egg masses. Inserra and Davis (1983), who observed *Hypoaspis* nr. *aculeifer* feeding on the egg masses of both cyst and root-knot nematodes could not determine if the mite fed only on the gelatinous matrix and not on the eggs. The inability of *H. calcuttaensis* to feed on the eggs embedded in the gelatinous matrix is indicative of its fluid-feeding behaviour. On the other hand, *T. putrescentiae*, which is better known as a stored grain pest, is generally considered to be omnivorous. These observations are consistent with those of Walter *et al.* (1988), who divided the predators of nematodes into two categories on the basis of their feeding behaviour i.e., fluid feeders (e. g., *Hypoaspis* spp.) and engulfers (e. g., *Tyrophagus zachvatkini*).

TABLE V - *Predation by different developmental stages of* T. putrescentiae *and* H. calcuttaensis *on second-stage juveniles of* M. javanica *(mean of 5 replicates).*

Mite species	Development stage	Per cent juvenile consumption after			Mean
		6 hrs	12 hrs	24 hrs	
T. putrescentiae	Larvae	35.4 (36.4)	57.8 (49.5)	76.2 (60.9)	56.5 (48.9)
	Nymphs	40.9 (39.7)	49.5 (44.7)	64.9 (53.7)	51.8 (46.0)
	Adults	43.4 (41.0)	78.4 (62.3)	76.4 (60.9)	66.1 (54.8)
H. calcuttaensis	Larvae	23.2 (28.4)	34.4 (35.8)	37.4 (37.7)	31.7 (33.9)
	Nymphs	54.8 (47.6)	72.7 (58.6)	76.2 (60.8)	67.9 (55.7)
	Adults	65.5 (54.1)	67.3 (55.1)	75.8 (60.6)	69.5 (56.6)
Mean		43.9 (41.2)	60.0 (51.0)	67.8 (55.8)	

Figures in parentheses are angular transformed values
Initial number of mites = 2 adults
Initial number of nematodes = 1000

	C.D. (5%)
Mites	= (2.9)
Period	= (2.1)
Mites x Period	= (5.0)

TABLE VI - *Influence of temperature on the predation by* T. putrescentiae *and* H. calcuttaensis *on the juveniles of* M. javanica *(mean of 10 replicates).*

Mite species	Per cent juvenile consumption after 24 hrs at			Mean
	20 ± 1 °C	25 ± 1 °C	30 ± 1 °C	
T. putrescentiae	70.4 (57.2)	68.1 (55.7)	55.7 (45.5)	64.8 (52.8)
H. calcuttaensis	78.8 (62.7)	97.7 (81.3)	94.7 (76.8)	90.4 (73.6)
Mean	74.6 (59.9)	82.9 (68.5)	75.2 (61.1)	

Figures in parentheses are angular transformed values
Initial number of mites = 2 adults
Initial number of nematodes = 1000

	C.D. (5%)
Mites	= (1.5)
Temperature	= (1.8)
Mites x Temperature	= (2.6)

The consumption of more dead than live nematodes by both the mite species can be attributed to nematode mobility. Predation was affected by temperature. Bilgrami (1994) observed that besides temperature, predation by *T. putrescentiae* was also influenced by agar concentrations, agar thickness and size of the test arena.

The present studies also revealed that all the acive stages of both the mites are capable of nematophagy, but the rate of predation by adults was greater. Larvae of *H. calcuttaensis* showed weak nematophagy probably because the mesostigmatid larvae are usually non-feeding and short-lived. Sell (1988) also reported that all the developmental stages of *Caloglyphus* sp. were able to predate upon different stages of root-knot nematodes. However, Rodriguez *et al.* (1962) observed that nymphs of *Macrocheles muscaedomesticae* preferred nematodes to housefly eggs, whereas adults preferred the latter.

Both *H. calcuttaensis* and *T. putrescentiae* were voracious feeders of nematodes in this study consuming as many as 811 and 726 juveniles *M. javanica* respectively, within the first 24 hrs. In terms of the total nematode biomass (wet weight) consumed, it can be expressed as 19.523 µmg/mite/day for *H. calcuttaensis* and 17.498 µg/mite/day for *T. petrescentiae*, based upon Andrassy's (1956) formula.

Agricultural soils harbour different types of nematodes. Hence, all kinds of nematodes are vulnerable to attack by predacious mites. The wide spread occurrence of nematophagous mites and their voracious feeding habits, as revealed by this study, indicate the potential role they play in the natural balance of nematode populations.

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