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Effect of Sodium Chloride on Biology of *Catenaria anguillulae*

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Growth studies of *Catenaria anguillulae* isolates in response to sodium chloride indicated that all the isolates grew in linseed oil-cake agar medium containing sodium chloride up to 1.0%. Medium with 1.5% sodium chloride, however, completely checked the growth of all the isolates. The size of zoosporangia greatly increased with abundant zoospore production in medium containing sodium chloride at 0.5%.

KEYWORDS: ***Catenaria anguillulae*, Endoparasite, Morphometrical, Sodium chloride**

Catenaria anguillulae is a facultative endoparasite of free living and plant parasitic nematodes (Sorokin, 1876; Esser and Ridings, 1973; Jaffee, 1986; Singh and Gupta, 1986; Singh *et al.*, 1996). It is widely distributed in soil (Barron, 1977; Persmark, 1995; Vaish and Singh, 2002). Its wide occurrence and parasitism on nematodes indicate, that this fungus plays important role in maintaining population of the nematodes in soil. It also indicates that the nematodes serve as food for *C. anguillulae*. Growth of *C. anguillulae* at a wide range of pH (Brichfield, 1960; Sayre and Keeley, 1969; Stirling and Platzer, 1978) maybe also one of the reasons for wide occurrence of this fungus in soil. Soils with different pH may also vary in the amount and quality of sodium chloride, which may also affect the biology of the fungus in soil. Isolates of *C. anguillulae* may show variation in tolerance and morphology of zoosporangia in response to sodium chloride concentration. In view of this, effect of different concentration of sodium chloride was studied on radial growth and morphological variations of 10 isolates of *C. anguillulae*. Observations on the same are described in this paper.

Materials and Methods

The isolation of *C. anguillulae* from soils was done by the method described by Singh *et al.* (1998). Purification of all the isolates tested (Table 1) was done from single sporangium zoospore culture following the method described by Singh (1989). Cultures of *C. anguillulae* were maintained on 0.3% beef extract agar medium (Beef extract 3 g; Agar 17 g; Distilled water 1,000 ml) by regular subculturing at an interval of 15 days. The cultures were always incubated at 30±1°C. For this study, sodium chloride was incorporated into 0.5% linseed oil-cake agar medium (Linseed oil-cake 5 g; Agar 15 g; Distilled water 1,000 ml). The experiment was conducted with six concentrations of sodium chloride viz., 0.5, 1.0, 1.5, 2.0, 2.5, and 5%. The sodium chloride was added at 5, 10, 15, 20, 25 and 50 g separately into one liter of 0.5% linseed oil-cake agar medium to make the above concentrations. The media were sterilized at 121°C for 20 minutes. Twenty ml of each medium were poured into 90 mm sterilized Petri dishes and inoculated with a fungal disc of 5 mm taken from the 10 day old culture of each isolate of *C. anguillulae*. Three replications were maintained for each treatment. Inoculation of Petri dishes containing linseed oil-cake agar medium without sodium chloride served as control. The inoculated Petri dishes were incubated at 30±1°C. Radial growth of the fungus was measured at intervals of three days upto 12 days. The experiment was conducted in a randomized block design and statistically analyzed.

Studies on morphometrical variations in 10 isolates in sodium chloride concentration were made using linseed oil-cake agar medium. Slides were prepared for each isolate from each medium in water/lacto phenol-cotton blue. The morphometrical characters like size of zoosporangia, size of discharge tubes and size of isthmuses were measured and recorded from these slides under a research microscope.

Results and Discussion

Effect of sodium chloride concentration on growth of *C. anguillulae*. The data on radial growth of different isolates of *C. anguillulae* in response to sodium chloride concentration in linseed oil-cake agar medium are presented in Table 1. It is evident from the observations that all the isolates grew at 0.5% and 1.0% sodium chloride concentration, however, the growth of all the isolates decreased with increasing concentration. None of the isolates grew when medium had 1.5% or more sodium chloride. At 0.5% sodium chloride concentration, maximum tolerance was recorded for the KP isolate which recorded

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Table 1. Radial growth of 10 isolates of *Catenaria anguillulae* on different concentration of sodium chloride on 12th day

Isolate	Concentrations of sodium chloride			Mean
	Average radial growth (mm)			
	0.5 %	1.0 %	Control	
VF	65.00	23.67	83.00	57.22
PA	35.33	12.00	51.00	32.77
KA	54.67	21.33	66.67	47.55
KP	46.33	16.67	53.00	38.66
CHP	32.33	9.33	60.33	33.99
KO	46.00	15.33	60.67	40.66
MA	45.00	15.00	60.00	40.00
MMT	42.33	14.33	52.67	36.44
SWP	37.33	14.00	60.67	37.33
GA	44.33	14.33	60.33	39.66
Mean	44.86	15.59	60.83	

	CD (P=0.05)	CD (P=0.01)
Isolates	0.409	0.524
Sodium chloride	0.258	0.331
Isolates × Sodium chloride	0.818	1.049

CD : critical difference

minimum reduction (12.58%) in radial growth followed by KA, MMT, VF, KO, MA, GA, PA and SWP (17.99, 19.63, 21.68, 24.17, 25.0, 26.52, 30.72 & 38.37%), while minimum tolerance was recorded for CHP isolate, which recorded maximum reduction (46.41%) in radial growth.

At 1.0% sodium chloride concentration radial growth of all the isolates, further decreased. The maximum radial growth was recorded for VF isolate followed by KA, KP, KO, MA, MMT, GA, SWP and PA isolates, while minimum growth was found for CHP isolate indicating that this isolate was more sensitive to sodium chloride.

Morphological variation in different isolates of *C. anguillulae* influenced by sodium chloride. The morphology of zoosporangia of all the isolates of *C. anguillulae* was greatly influenced by sodium chloride at 0.5% and 1.0% concentration (Table 2, Figs. 1 and 2). In general, the zoosporangia of all the isolates increased in size, in sodium chloride amended media.

VF isolate, produced typically tubular or elongate elliptical or elliptical zoosporangia on linseed oil-cake agar medium. In 0.5% sodium chloride, this isolate produced

Table 2. Size of sporangia, discharge tubes and isthmuses of different isolates of *Catenaria anguillulae* at different sodium chloride concentration on linseed oil-cake agar medium

Isolate		Size (μm)		
		0.0 % NaCl	0.5% NaCl	1.0% NaCl
VF	Sporangium	57~213 × 6~21	61~122 × 15~67	158~550 × 20~54
	Discharge tube	109~530 × 3	91~204 × 9~22	-
	Isthmus	9~106 × 6	15~61 × 9	6~18 × 6
PA	Sporangium	12~24 × 15~33	18~36 × 21~43	40~140 × 30~80
	Discharge tube	12~64 × 3	36~103 × 3~6	-
	Isthmus	15~51 × 6	30~76 × 6	42~91 × 6
KA	Sporangium	61~213 × 6~42	33~115 × 61~125	54~91 × 67~106
	Discharge tube	67~356 × 3~6	189~823 × 6~22	280~597 × 6~12
	Isthmus	9~67 × 6	6~51 × 6	6~36 × 6
KP	Sporangium	27~39 × 27~33	282~472 × 25~70	201~341 × 25~91
	Discharge tube	228~564 × 3	-	-
	Isthmus	15~51 × 6	9~61 × 6	6~24 × 6
CHP	Sporangium	36~61 × 15~24	24~47 × 36~61	85~190 × 20~38
	Discharge tube	33~219 × 3~6	15~61 × 9~18	-
	Isthmus	24~106 × 6	21~61 × 6	6~39 × 6
KO	Sporangium	24~67 × 15~18	15~36~18~61	30~76 × 15~39
	Discharge tube	97~372 × 3	6~45 × 6~12	-
	Isthmus	15~91 × 6	9~51 × 6	9~54 × 6
MA	Sporangium	30~45 × 36~48	137~422 × 30~72	67~230 × 36~79
	Discharge tube	67~317 × 3~9	-	-
	Isthmus	6~45 × 6	12~39 × 6	6~45 × 9
MMT	Sporangium	27~45 × 30~45	76~539 × 20~67	115~488 × 36~91
	Discharge tube	67~399 × 3~9	-	-
	Isthmus	15~82 × 6	15~45 × 6	6~33 × 9
SWP	Sporangium	45~91 × 15~36	106~415 × 30~73	103~268 × 14~36
	Discharge tube	36~280 × 3	-	-
	Isthmus	6~106 × 6	6~36 × 6	6~30 × 6
GA	Sporangium	54~143 × 6~18	30~85 × 24~84	135~320 × 20~76
	Discharge tube	18~262 × 3	54~280 × 6~12	-
	Isthmus	15~91 × 6	12~45 × 6~9	6~33 × 6

bold zoosporangia that were broadly elliptical and wider in width and frequency of the tubular zoosporangia was rare. Discharge tubes of zoosporangia in 0.5% sodium chloride linseed oilcake agar were much wider as compared to medium without sodium chloride. The zoosporangia were packed with zoospores, which were released only when placed in water within 1/2 hour. Zoosporangia of this isolate in 1.0% sodium chloride were round/pyriform at the base with elongated neck.

The zoosporangia of GA isolate also recorded more or less similar morphology at 0.5% and 1.0% sodium chloride concentration.

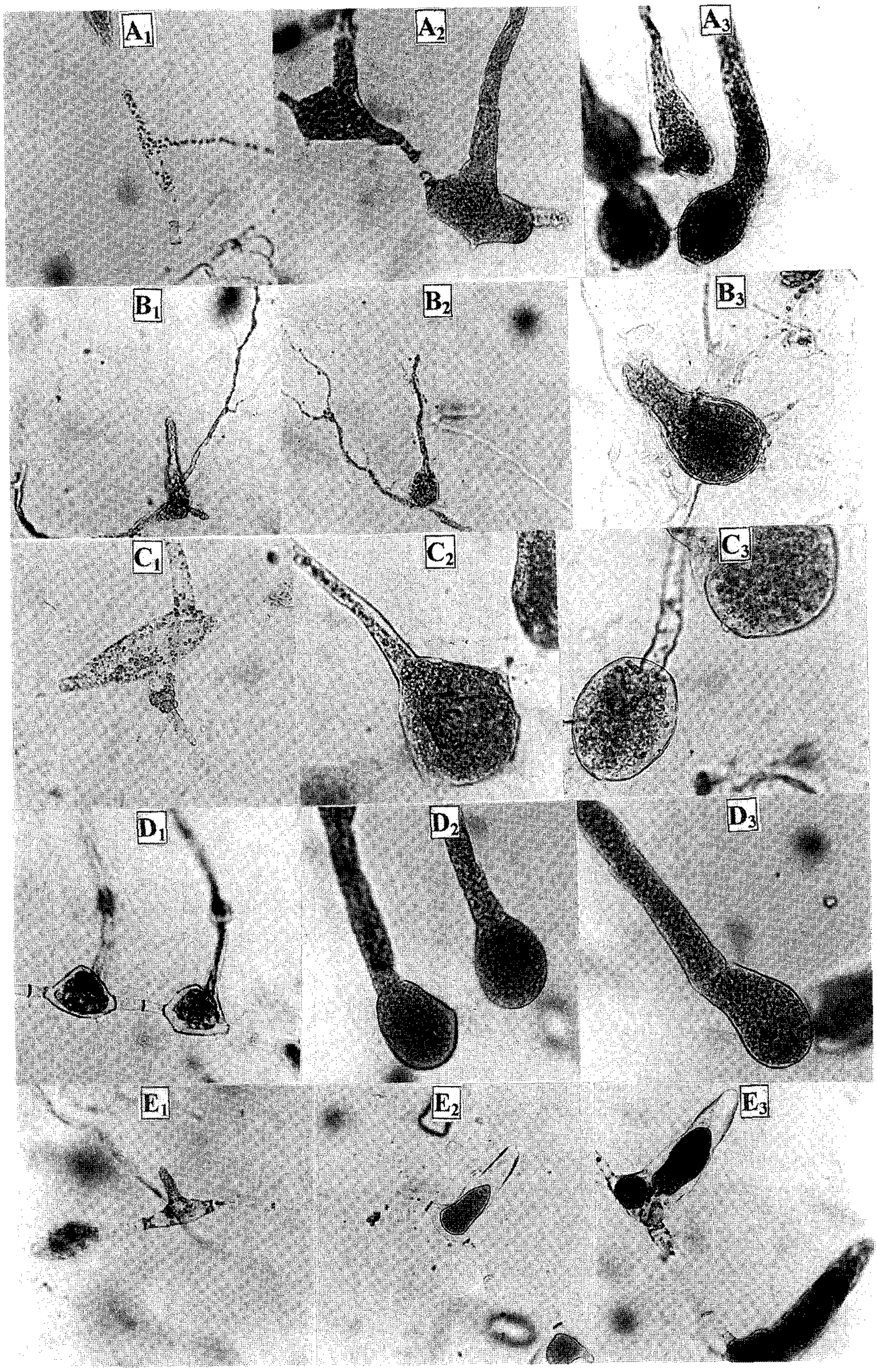


Fig. 1. Variations in sporangial morphology of different isolates of *Catenaria anguillulae* as influenced by different sodium chloride concentrations (×220).
A_1: Morphology of sporangia of isolate VF on linseed oil-cake agar medium, A_2: Morphology of sporangia of isolate VF on 0.5% NaCl amended linseed oil-cake agar medium, A_3: Morphology of sporangia of isolate VF on 1.0% NaCl amended linseed oil-cake agar medium, B_1: Morphology of sporangia of isolate PA on linseed oil-cake agar medium, B_2: Morphology of sporangia of isolate PA on 0.5% NaCl amended linseed oil-cake agar medium, B_3: Morphology of sporangia of isolate PA on 1.0% NaCl amended linseed oil-cake agar medium, C_1: Morphology of sporangia of isolate KA on linseed oil-cake agar medium, C_2: Morphology of sporangia of isolate KA on 0.5% NaCl amended linseed oil-cake agar medium, C_3: Morphology of sporangia of isolate KA on 1.0% NaCl amended linseed oil-cake agar medium, D_1: Morphology of sporangia of isolate KP on linseed oil-cake agar medium, D_2: Morphology of sporangia of isolate KP on 0.5% NaCl amended linseed oil-cake agar medium, D_3: Morphology of sporangia of isolate KP on 1.0% NaCl amended linseed oil-cake agar medium, E_1: Morphology of sporangia of isolate CHP on linseed oil-cake agar medium, E_2: Morphology of sporangia of isolate CHP on 0.5% NaCl amended linseed oil-cake agar medium, E_3: Morphology of sporangia of isolate CHP on 1.0% NaCl amended linseed oil-cake agar medium.

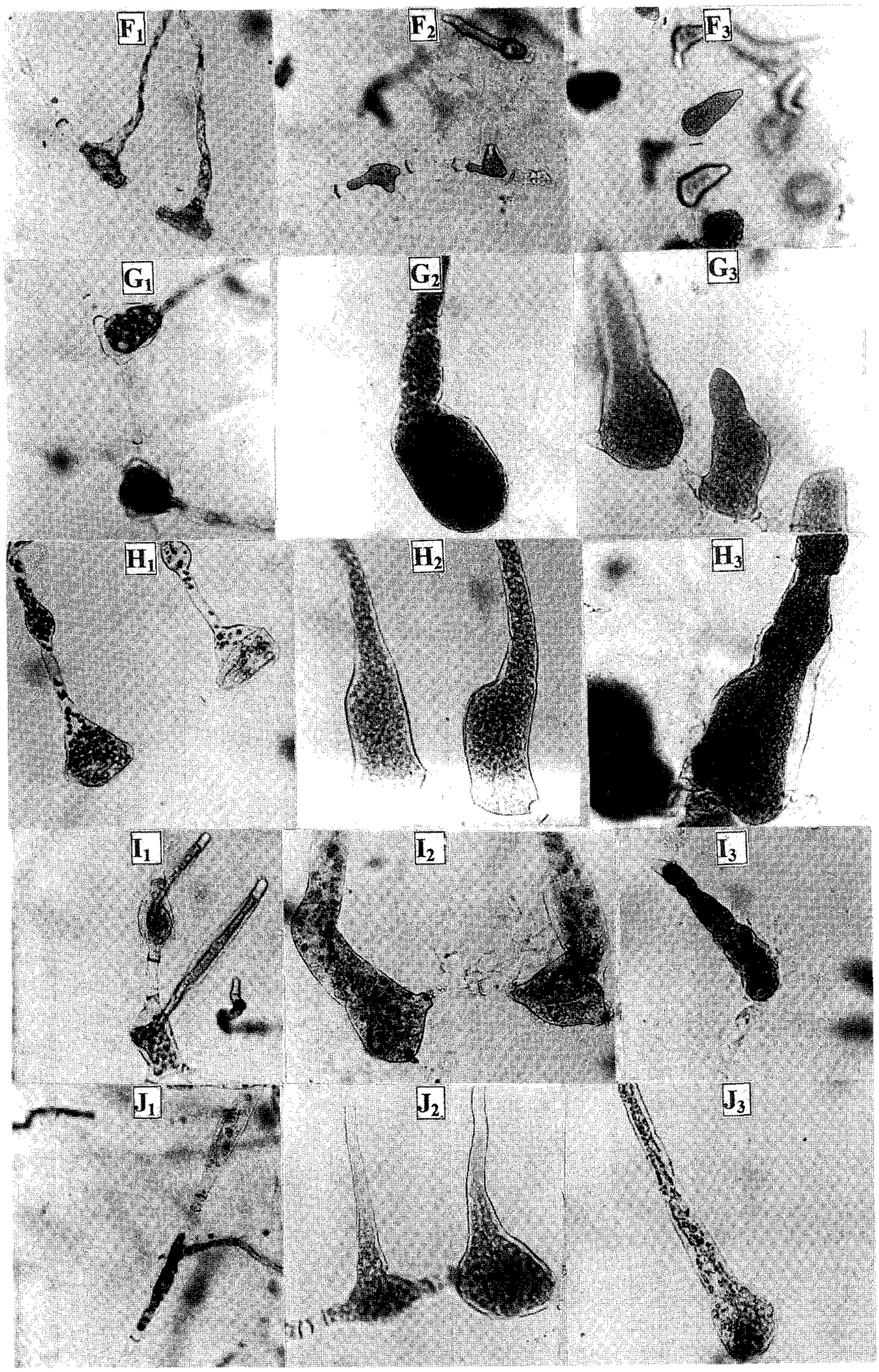
KA isolate producing elongate, broadly elliptical or occasionally tubular zoosporangia on linseed oil-cake agar produced almost round and larger zoosporangia with narrow discharge tubes at 0.5% sodium chloride. In 1.0% sodium chloride also, this isolate produced round zoosporangia with narrow discharge tubes. In SWP isolate, zoosporangia were conical in medium containing 0.5% and 1.0% sodium chloride.

Two isolates CHP and KO producing elliptical or vertically elongate zoosporangia produced abundant resting sporangia with characteristic pips. The zoosporangia in both the isolates were mostly conical in 0.5% sodium chloride. In 1.0% sodium chloride also the resting sporangia were more abundant and mostly conical in shape. Three isolates KP, MA and MMT producing spherical or sub spherical zoosporangia produced larger zoosporangia with long necks with even distribution of developing zoospores upto the neck. In 0.5% sodium chloride the zoosporangia of KP isolate were spherical at base and terminated into long necks. In 1.0% sodium chloride the zoosporangia were sub spherical at base with long beak showing uniform width. In MA and MMT isolates also the zoosporangia were greatly enlarged in medium containing 0.5 and 1.0% sodium chloride. However, in 1.0% sodium chloride, zoosporangia were mostly conical in both the isolates. In these zoosporangia, although zoospore development occurred but release of zoospores could be observed only when zoosporangia were placed in fresh water.

Observations on the effect of sodium chloride concentrations on growth of *C. anguillulae* (Table 1) indicated that the isolates exhibit sodium chloride tolerance up to 1.0% when grown on supplemented medium. The study also revealed that the isolates varied in their degree of tolerance. From the observations it is postulated that this fungus can grow in alkaline/saline soils. The variation in the degree of tolerance to sodium chloride may be genetically dependent.

The change in morphology and size of zoosporangia as well as increased zoospore production in a medium containing sodium chloride may be attributed to the role of sodium as an essential/beneficial element or increased uptake of nutrients in the presence of sodium chloride (Table 2). Although sodium has been recognized as essential element for some plants, its similar role needs to be established in fungi. However, the role of sodium chloride in the increased reproductive biology of *C. anguillulae* is established from this study. The width of wall of zoosporangia of *C. anguillulae* in the sodium chloride medium (Figs. 1 and 2) is increased as compared to zoosporangia developed in linseed oil-cake agar medium without sodium chloride. This corroborates with the earlier observa-

Fig. 2. Variations in sporangial morphology of different isolates of *Catenaria anguillulae* as influenced by different sodium chloride concentrations (×220).
F_1: Morphology of sporangia of isolate KO on linseed oil-cake agar medium, F_2: Morphology of sporangia of isolate KO on 0.5% NaCl amended linseed oil-cake agar medium, F_3: Morphology of sporangia of isolate KO on 1.0% NaCl amended linseed oil-cake agar medium, G_1: Morphology of sporangia of isolate MA on linseed oil-cake agar medium, G_2: Morphology of sporangia of isolate MA on 0.5% NaCl amended linseed oil-cake agar medium, G_3: Morphology of sporangia of isolate MA on 1.0% NaCl amended linseed oil-cake agar medium, H_1: Morphology of sporangia of isolate MMT on linseed oil-cake agar medium, H_2: Morphology of sporangia of isolate MMT on 0.5% NaCl amended linseed oil-cake agar medium, H_3: Morphology of sporangia of isolate MMT on 1.0% NaCl amended linseed oil-cake agar medium, I_1: Morphology of sporangia of isolate SWP on linseed oil-cake agar medium, I_2: Morphology of sporangia of isolate SWP on 0.5% NaCl amended linseed oil-cake agar medium, I_3: Morphology of sporangia of isolate SWP on 1.0% NaCl amended linseed oil-cake agar medium, J_1: Morphology of sporangia of isolate GA on linseed oil-cake agar medium, J_2: Morphology of sporangia of isolate GA on 0.5% NaCl amended linseed oil-cake agar medium, J_3: Morphology of sporangia of isolate GA on 1.0% NaCl amended linseed oil-cake agar medium.



tions made on thickness of cell wall in response to sodium chloride in archaeobacteria (Prescott *et al.*, 1996).

References

Barron, G. L. 1977. The nematode destroying fungi. *Topics in Mycobiology* No. 1, 140 P Guelph: Canadian Biological Publication.

Brichfield, W. 1960a. Growth studies of *Catenaria* sp. infecting nematodes. *Phytopathology* **50**: 629.

Esser, R. P. and Ridings, W. H. 1973. Pathogenicity of selected nematodes by *Catenaria aguillulae. Proceedings of the soil and Crop Science Society of Florida* **33**: 60-64.

Jaffee, B. A. 1986. Parasitism of *Xiphinema rivesi* and *Xiphinema americanum* by zoosporic fungi. *Journal of Nematology* **18**: 87-95.

Persmark, L. Mondoza, N. M. and Jansson, H. B. 1995. Nematophagous fungi from agricultural soils of Central America. *Nematropica* **25**: 117-124.

Prescott, L. M., Harley, J. P. and Klein, D. A.,1996 Microbiology. Third edition. Wm. C. Pub. Pp 486

Sayre, R. M. and Keeley, L. S. 1969. Factors influencing *Catenaria anguillulae* infections in a free living and a plant-parasitic nematode. *Nematologica* **15**: 492-502.

Singh, K. P. 1989. Artificial culture of *Cataenaria anguillulae* from monosporangial zoospores. *Mycological Research* **92**: 107.

_____ and Gupta, P. 1986. Observataions on *Catenaria anguillulae* parasitic on *Heterodera sorghi*. *Advances in Biological Research* **4**: 240-250.

_____, Bandyopadhyay, P. Stephen, R. A. Vaish, S. S. and Kumar, Makesh T. 1998. Techniques for selective isolation, semiquantification and rapid virulence testing of *Catenaria anguillulae*. *Mycological Research* **102**: 658-660.

_____, Stephen, R. A. and Vaish, S. S. 1996. Pathogenicity and development of *Catenaria anguillulae* on some nematodes. *Mycological Research* **100**: 1204-1206.

Sorokin, N. 1876. Note sur les vegetaux parasites des *Anguillulae. Annales des Sciences Naturalles, Botanique Ser.* 6, **4**: 62-71.

Stirling, A. M. and Platzer, E. G. 1978. *Catenaria anguillulae* in the mermithid nematode *Romanomermis culicivorax. Journal of Invertibrate Pathology* **32**: 348-354.

Vaish, S. S. and Singh, K. P. 2002. Distribution of *Catenaria anguillulae* Sorokin, a facultative endoparasite of nematodes in soils from different locations of India. *World Journal of Microbiology and Biotechnology* **18**: 65-67.