*J. Mycopathol. Res. 41(2) : 251-254 (2003)*


# *In vitro* variability in virulence of *Catenaria anguillulae* Sorokin

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Pathogenicity of ten isolates of *Catenaria anguillulae* was tested *in vitro* against *Xiphinema basiri, Hoplolaimus indicus* and *Hemicriconemoides mangiferae.* Of all the isolats of *C. anguillulae,* VF isolate was found to be most virulent. *Xiphinema basiri* was found to be most susceptible.

**Key Words :** *Catenaria anguillulae, in vitro,* pathogenicity, variability, virulence

## INTRODUCTION

*Catenaria anguillulae* is a endoparasite of free living and plant parasitic nematode. It is widely distributed in soil (Barron, 1977 ; Persmark, 1995 ; Vaish and Singh, 2002). Its wide occurrence and parasitism on nematodes indicate that fungus plays important role in maintaining population of the nematodes in soil. However, there are several reports on parasitism of plant parasitic nematodes by *C. anguilillae* (Boosalis and Mankau, 1965 ; Sayre and Keeley, 1969 ; Esser and Ridings, 1973 ; Jaffee, 1986 ; Singh *et al.,* 1996). Some workers have concluded that isolates of *C. anguillulae* differ in virulence (Esser and Riding, 1973 ; Jaffee and Shaffer, 1987 ; Voss, 1988). Stirling and Platzar (1978) have observed that an isolate of *C. anguillulae* from *Romanomermis culcivorax* readily infected *R. culcivorax,* whereas, another isolate from *Hemicycliophira arenaria* has been unable to infect *R. clucivorax.*

Vaish *et al.*, (1997) while working on five isolats of *C. anguillulae* have found all the isloates to be virulent, however, degree of virulence varies against second stage juveniles of *Heterodera cajani.* Nematode species are known to differ in their relative susceptibility to *Catenaria* infection. Sayre and Keeley (1969) have reported that *Panagrellus redivivus* is more susceptible than *Ditylenchus dipsaci.* Jaffee and Shaffer (1987) have reported parasitism of *Xiphinema americanum* and *X. rivesi* by *C. anguillulae* in both soil and soil soultion. However, they have found *X. rivesi* to be more susceptible to *C. anguillulae* than *X. americanum.* Because of its wide occurrence under different seasons through out the year, there is possiblility that the isolates of *C. anguilullae* differ in their biology particularly in relation to virulence. If so, studies on the above aspect become more important from academic as well as applied view points. This requires detail observations on virulence of a large number isolates of *C. anguillulae* against nematodes.

## MATERIALS AND METHODS

### *Isolation of Catenaria anguillulae*

Soil samples were collected from different locations for the isolation of *Catenaria anguillulae* : vegetable farm, BHU, Koirajpur, Chitaipur, Mahamanapuri, Sewapuri, (Varanasi), Tejpura (Ghajipur), Kachagawan (Janupur), Maudaha (Hamirpur), Research Farm, C.S.A. University of Agriculture & Technology, Kanpur and Research Farm, G. B. Pant University of Agriculture & Technology, Pantnagar.

The isolation of *C. anguillulae* from soils was done by the method described by Singh et al. (1998). Purification of all the isolates 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 1000 ml) by regular subculturing at an

interval of 15 days. The cultures were always incubated at 30 ± 1°C.

***Collection of plant parasitic nematodes***

Population of *Xiphinema basiri* was collected from the soil around the roots of croton (*Codiaeum variegatum* L. Blume) plants, while *Hoplolaimus indicus* was collected from the soil around the roots of banana (*Musa paradisiaca*) plants and population of *Hemicriconemoides mangeferae* from the soil around the roots of mango (*Mangifera indica*) trees of Horticulture Research Farm, B.H.U. Varanasi.

***Collection of nematodes from soil***

Soil samples were collected in polyethylene bags from different locations as given above and brought to the laboratory. The soil samples were thoroughly mixed to make homogeneous soil. From this 500 g of soil was placed in a bucket, suspended in water and agitated throughly to obtain a homogenous suspension. The suspension was allowed to remain undisturbed for few minutes to enable the larger size particles of the soil to settle down, and then the supernatant liquid containing nematodes was passed through a set of sieves i.e. 60, 100, 200 and 300 mesh sieves. The residues from 60 mesh was directly examined under the stereoscopic binocular microscope for bigger nematodes, whereas, the suspension obtained from 100, 200 and 300 mesh sieves were passed through double layers of tissue paper supported on the wire mesh on the several petri dishes.

After a period of 36-38 hrs., when water on the tissue paper had practically evaporated the water from the petri dishes containing nematodes was collected into a beaker and examined under stereoscopic binocular microscope to identify and count the desired species of nematodes.

***In vitro pathogenicity test of C. anguillulae against some plant parasitic nematodes***

The pathogenicity test of the ten isolates of C. *anguillulae* was conducted against *Xiphinema baseri, Hoplolaimus indicus* and *Hemicriconemoides mangiferae* following the method described by VoB and Wyss (1990). The nematode populations were collected from the soil suspensions in large numbers in cavity blocks. For this purpose, 20 nematodes of each mematode species were added into several cavity blocks containing 2 ml of sterilized distilled water, after five washing with sterilized distilled water. Then a fungal disc (3 mm diam.) from 10 day old cultures of each isolate was inoculated into each cavity block as inoculum containing 20 nematodes. After this the cavity blocks were incubated at 30±1°C for infection. After 24 of incubation, the inoculated fungal disc was removed aseptically. The observations on infections/mortality were made daily up to six days after inoculation. Each treatment was replicated three times. The experiment was repeated three times and the percentage of mortality of nematodes was calculated from the pooled data. The experiment was conducted in Randomized block design and statistically analyzed.

## RESULTS AND DISCUSSION

***In vitro pathogenicity test of 10 isolates of C. anguillulae against Xiphinema basiri***

Observations on *in vitro* pathogenicity test of 10 isolates of *C. anguillulae* against *Xiphinema basiri* are presented in Table 1. It is apparent from the data that percentage mortality of *X. basiri* significantly differed with different isolates of *C. anguillulae* which clearly indicated that the isolates taken in the present study varied greatly in their virulance. The mamimum mortality was caused by VFd and GA isolates, however, their percentage mortality did not differ significantly for KA, KP and CHP isolates. Isolate PA, MMT and MA caused significantly lower per cent of mortality in *X. basiri.* In general, the percentage mortality increased significantly with increasing time of incubation.

***In vitro pathogenicity test of 10 isolates of C. anguillulae against Hoplolaimus indicus***

Pathogenicity test of 10 isolates of *C. anguillulae* against *Hoplolaimus indicus* (Table 2) clearly revealed that isolates, VF, GA, KA and SWP were more virulent than other isolates as they caused sig-

nificantly higher mortality on 6th day. The percentage mortality caused by other isolates was non significant as compared to control except KP isolate.

**Table 1 :** *In vitro* pathogenicity test of 10 isolates of *Catenaria anguillulae* on *Xiphinema basiri.*

| Isolate | Incubation period (Day) Mortality (%) | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| VF | 5.00 | 16.66 | 28.33 | 68.33 | 86.66 | 100.00 | 50.83 |
| PA | 0.00 | 1.66 | 5.00 | 13.33 | 20.00 | 28.33 | 11.38 |
| KA | 5.00 | 18.33 | 23.33 | 65.00 | 78.33 | 95.00 | 47.49 |
| KP | 1.66 | 13.33 | 20.00 | 60.00 | 78.33 | 93.33 | 44.44 |
| CHP | 5.00 | 13.33 | 20.00 | 60.00 | 78.33 | 93.33 | 44.99 |
| KO | 1.66 | 13.33 | 20.00 | 46.66 | 81.66 | 91.66 | 42.49 |
| MA | 1.66 | 13.33 | 20.00 | 46.66 | 56.66 | 70.00 | 34.71 |
| MMT | 5.00 | 13.33 | 20.00 | 25.00 | 41.66 | 56.66 | 26.94 |
| SWP | 5.00 | 15.00 | 20.00 | 46.66 | 78.33 | 91.66 | 42.77 |
| GA | 5.00 | 16.66 | 28.33 | 68.33 | 85.00 | 100.00 | 50.55 |
| Control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Mean | 3.18 | 12.26 | 18.63 | 45.45 | 69.39 | 74.54 | |

| | CD at 5% level | CD at 1% level |
|---|---|---|
| Isolates | 5.149 | 6.751 |
| Days | 3.256 | 4.071 |
| Isolates x Days | 10.298 | 13.503 |

Table 2. *In vitro* pathogenicity test of 10 isolates of *Catenaria anguillulae* on *Hoplolaimus indicus.*

| Isolate | Incubation period (Day) Mortality (%) | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| VF | 0.00 | 0.00 | 0.00 | 6.66 | 8.00 | 13.33 | 4.66 |
| PA | 0.00 | 0.00 | 0.00 | 0.00 | 1.66 | 1.66 | 0.55 |
| KA | 0.00 | 0.00 | 0.00 | 3.33 | 6.66 | 11.66 | 3.60 |
| KP | 0.00 | 0.00 | 0.00 | 3.33 | 6.66 | 6.66 | 2.77 |
| CHP | 0.00 | 0.00 | 0.00 | 0.00 | 3.33 | 3.33 | 1.11 |
| KO | 0.00 | 0.00 | 0.00 | 1.66 | 1.66 | 3.33 | 1.10 |
| MA | 0.00 | 0.00 | 0.00 | 0.00 | 3.33 | 3.33 | 1.11 |
| MMT | 0.00 | 0.00 | 0.00 | 0.00 | 1.66 | 1.66 | 0.55 |
| SWP | 0.00 | 0.00 | 0.00 | 0.00 | 4.66 | 11.66 | 3.05 |
| GA | 0.00 | 0.00 | 0.00 | 5.00 | 8.00 | 11.66 | 4.11 |
| Control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Mean | 0.00 | 0.00 | 0.00 | 1.81 | 4.32 | 6.20 | |

| | CD at 5% level | CD at 1% level |
|---|---|---|
| Isolates | 1.645 | 2.158 |
| Days | 1.040 | 1.301 |
| Isolates x Days | 3.291 | 4.316 |

*In vitro patahogenicity test of 10 isolates of C. anguillulae against Hemicriconemoides mangiferae Hemicriconemoides mangiferae*was found to be very tolerant to *Catenaria anguillulae* infection. However, maximum percentage mortality of this nematode (11.66%) was recorded with VF isolate on 6th day of incubation. PA isolate failed to infect this nematode, hence there was no mortality. The other isolates viz., KA, KP, CHP, GA, KO, MA, MMT and SWP caused lower mortality of this nematode (Table 3).

**Table 3 :** *In vitro* pathogenicity test of 10 isolates of *Catenaria anguillulae* on *Hemicriconemoides mangiferae.*

| Isolate | Incubation period (Day) Mortality (%) | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| VF | 0.00 | 0.00 | 0.00 | 3.33 | 6.66 | 11.66 | 3.60 |
| PA | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| KA | 0.00 | 0.00 | 0.00 | 1.66 | 3.33 | 8.33 | 2.22 |
| KP | 0.00 | 0.00 | 0.00 | 3.33 | 5.00 | 8.33 | 2.77 |
| CHP | 0.00 | 0.00 | 0.00 | 1.66 | 3.33 | 8.33 | 2.22 |
| KO | 0.00 | 0.00 | 0.00 | 1.66 | 3.33 | 6.66 | 1.94 |
| MA | 0.00 | 0.00 | 0.00 | 1.66 | 3.33 | 6.66 | 1.94 |
| MMT | 0.00 | 0.00 | 0.00 | 1.66 | 3.33 | 5.00 | 1.66 |
| SWP | 0.00 | 0.00 | 0.00 | 0.00 | 3.33 | 3.33 | 1.11 |
| GA | 0.00 | 0.00 | 0.00 | 1.66 | 3.33 | 8.33 | 2.22 |
| Control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Mean | 0.00 | 0.00 | 0.00 | 1.51 | 3.17 | 6.81 | |

| | CD at 5% level | CD at 1% level |
|---|---|---|
| Isolates | 1.403 | 1.840 |
| Days | 0.887 | 1.109 |
| Isolates x Days | 2.807 | 3.680 |

Pathogenicity test of *C. anguillulae* against different plant parasitic nematodes (Tables 1-3 and Fig. 1) indicate that isolates differed significantly in their virulence. Among all the isolates tested, VF isolate showed maximum virulence against all the nematodes included in this study indicating that this isolate was polyvirulent, whereas, PA was found to be least virulent. Of all the plant parasitic nematodes *Xiphinema basiri* was the most susceptible showing 100% infection on sixth day of inoculation with VF and GA isolates. The other isolates recorded lower percentage of infection as compared to VF and GA isolates. *Hoplolaimus indicus* and *Hemicriconemoides mangiferae* showed lower percentage of infection and mortality. The higher susceptibility of *X. basiri* to *C. anguillulae* isolates as compared to *H. indicus* and *H. mangiferae* is in conformity with the observations of Mankau and Sweeney (1963) and Singh *et al.* (1996). Variation in the degree of virulence of *C. anguillulae* has been reported earlier by some sorkers (Esser and Riding, 1973 ; Jaffee and Shaffer, 1987 ; Voss, 1988).

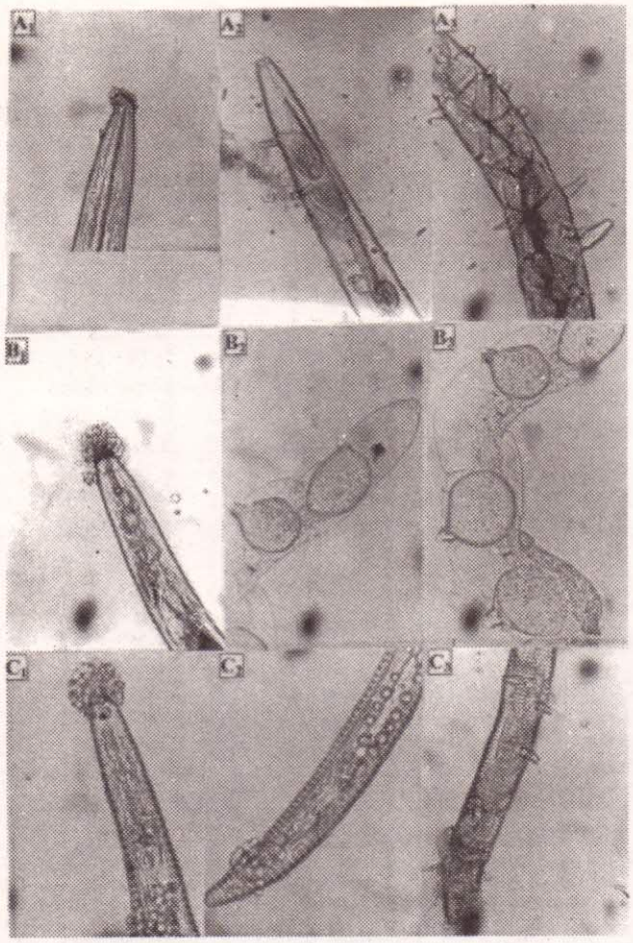

**Fig. 1 :** Nematodes parasitized by V. F. isolate of *Catenaria anguillulae*.
$A_1$ : Colonization of zoospore of *C. anguillulae* on mouth region of *Xiphinema basiri*. (500X). $A_2$ : Developed sporangia and discharge tubes of *C. anguillulae* (500X) ; $A_3$ : Developed sporanigia in double rows of *C. anguillulae* in the body of *Xiphinema basiri*. (750X) ; $B_1$ : Colonization of zoospores of *C. anguillulae* at mouth region of *Hoplolaimus indicus*. (500X) ; $B_2$ : Developed sporangia with small discharge tubes of *C. anguillulae* in the body of *Hoplolaimus indicus*. (750X) ; $B_3$ : Developed sporangia connected with isthumuses of *C. anguillulae* (750X) ; $C_1$ : Colonization of zoospores of *C. anguillulae* at mouth region of *Hemicriconemoids mangiferae*. (500X) ; $C_2$ : Colonization of zoospores of *C. anguillulae* at the anal region of *Hemicriconemoids mangiferae*. (500X) ; $C_3$ : Developed sporangia with discharge tubes of *C. anguillulae* in the body of *Hemicriconemoids mangiferae*. (500X) ;

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*(Accepted for publication September 03 2003)*