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Effect of Temperature on Biology of Different Isolates of *Catenaria anguillulae*

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Growth of ten isolates of *Catenaria anguillulae* on linseed oil-cake agar medium was studied at 10, 13, 15, 20, 25, 30, 35, 40, 44 and 46°C. The cardinal temperatures of these isolates were also determined. Observations clearly revealed that the isolates differed in their temperature requirements. Isolate GA was found to grow best at 40°C, whereas VF isolate showed best growth at 35 and 40°C. Isolates PA, KA, CHP, KO, MA and SWP grew best at 35°C. The other isolates (KP and MMT) showed their best growth at 30°C. Based on radial growth, isolates were categorized as fast growing, medium growing and slow growing. Studies on effect of different temperatures on pathogenicity was made using only three isolates: VF, KP and MA against *Xiphinema basiri*. VF isolate caused maximum infection at 40°C, whereas KP and MA isolates caused maximum infection at 30°C. The morphology of sporangia varied with temperature in isolates producing elongate-elliptical or filamentous sporangia. In VF isolate the sporangia were largely filamentous to elongate elliptical at 30°C. The width of the sporangia increased with increasing and decreasing temperatures. At 44°C the sporangia of this isolate were mostly broadly elliptical or spherical. This clearly indicates that sporangia vary in morphology with temperature. From the morphometrical studies it was understood that sporangial morphology was more consistent and reliable for grouping of isolates. Based on the morphology of sporangia the isolates of *C. anguillulae* were characterized in three groups viz., filamentous/elongate elliptical (VF, KA, GA and SWP), spherical (PA, KP, MA and MMT) and vertically elongate sporangia (CHP and KO).

KEYWORDS: ***Catenaria anguillulae*, Endoparasite, Morphometrical, Pathogenecity, Temperature**

Catenaria anguillulae Sorokin is a facultative endoparasite of nematodes (Sorokin, 1876; Jaffee, 1986; Singh and Gupta, 1986; Esser and Ridings, 1973; Singh *et al.*, 1996). The success of any biocontrol agent depends on its ability to survive at a wide range of temperature in the ecosystem. Temperature in relation to growth of *C. anguillulae* has been studied by few workers (Brichfield, 1960a; Stirling and Platzer, 1978; VoB and Wyss, 1990; Stephen, 1992). However, in most of the cases, earlier workers have studied the temperature requirement of a single isolate except VoB and Wyss (1990) who studied temperature requirement of 19 isolates. From their observations it appears that isolates varied in their temperature requirements to some extent. Occurrence of *C. anguillulae* throughout the year in soil (Vaish and Singh, 2002) particularly in hot summer when maximum day temperature casually going up to 45°C indicate the possibility of some isolates showing thermotolerant nature. Observations on this kind of variability will be helpful in differentiating the isolates of *C. anguillulae*. The temperature may also affect infectivity of *C. anguillulae* which has been studied by few workers (Sayre and Keeley, 1969; VoB and Wyss, 1990). Several workers have observed zoosporangia of different shapes ranging from spherical, elliptical, oval, triangular, pyriform, subpyriform, globose and elongate (Couch, 1945; Sparrow, 1960; Karling, 1977; Olson and Reichle, 1978; Stirling and Platzer, 1978; Singh and Gupta, 1986). However, morphological variations in response to temperatures have not been studied by earlier workers. Recently Gupta and Singh (2002) studied effect of sodium chloride on biology of *C. anguillulae* and reported morphological variations in different isolates of *C. anguillulae*. Similarly temperature may also affect size and morphology of sporangia of this fungus. In view of the above, effect of temperature on growth, pathogenecity and morphological variations of 10 isolates of *C. anguillulae* was studied and the observations are reported in this paper.

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Materials and Methods

Collection of isolates of *C. anguillulae*. Soil samples were collected from different locations for the isolation of *C. anguillulae*: Vegetable farm, B.H.U., Research Farm, G. B. Pant University of Agriculture & Technology, Pantnagar, Kachagawan (Jaunpur), Research Farm, C.S.A. University of Agriculture & Technology, Kanpur, Chitaipur, Koirajpur, Maudaha (Hamirpur), Mahamanapuri, Sewapuri, (Varanasi) and Ghazipur. The isolates were designated on the basis of places: VF, PA, KA, KP, CHP, KO, MA, MMT, SWP and GA.

Effect of different temperatures on growth of *C. anguillulae*. 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 spo-

rangium 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. The growth of *C. anguillulae* at different temperatures was studied on linseed oil-cake agar medium (Linseed oil-cake 5 g; Agar 15 g; Distilled water 1000 *ml*). The medium was prepared, sterilized at 15 psi for 20 minutes and poured into several sterilized 90 mm Petri dishes. A block of 5 mm diameter of different isolates of *C. anguillulae* taken from the periphery of 10 day old culture was inoculated separately into each of several Petri dishes. The inoculated Petri dishes were incubated at different temperatures 10, 13, 15, 20, 25, 30, 35, 40, 44 and 46°C. Three replications were maintained for each isolate and temperature. Radial growth of the fungus was measured at intervals of three days until some Petri dishes were fully covered by fungal growth or up to 12 days. The experiment was repeated thrice. The experiment was conducted in Randomized block design and statistically analyzed.

Effect of different temperatures on pathogenicity of *C. anguillulae* against *X. basiri*. *In vitro* pathogenicity test of the three selected isolates VF, KP and MA of *C. anguillulae* was conducted against *X. basiri* at different temperatures : 15, 20, 25, 30, 35 and 40 degree celsius at pH 7.0. For this purpose 20 adults of *X. basiri* washed five times with sterilized water were added into each of several cavity blocks containing 2 *ml* of sterilized distilled water (pH 7.0). Three mm fungal disc from 10 day old cultures of the selected isolates was inoculated into each cavity block separately as inoculum. After 24 hours of incubation, the inoculated fungal disc was removed aseptically. The observations on infection were made on 1st, 2nd, 3rd and 4th day 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.

Morphometrical variations in sporangia, discharge tubes and isthmuses of *C. anguillulae*. For studies on morphometrical variations in selected isolates of *C. anguillulae* in relation to different temperatures, isolates were grown on linseed oil-cake agar medium (LAM). Slides were prepared for each isolate from each temperature in lacto phenolcotton blue. The morphometrical characters like size of sporangia, size of discharge tubes and size of isthmuses were measured and recorded from these slides under a research microscope.

Results and Discussion

None of the isolates grew at 10°C. VF isolate recorded significantly more radial growth than other isolates. From the data on radial growth of different isolates at 13°C to 25°C, it is clear that growth rate/radial growth may not be utilized for differentiation of isolates at these temperatures (Table 1). In general, growth of all the isolates significantly increased with increasing temperature (13~ 25°C).

A marked increase in radial growth of all the isolates was recorded at 30°C (Table 2). At this temperature, the

Table 1. Radial growth of 10 isolates of *Catenaria anguillulae* on linseed oil-cake agar medium on 12 day at different temperatures

Isolate	Radial growth (mm)					Mean
	Temperature (°C)					
	13	15	20	25	30	
VF	13.00	18.00	26.33	41.66	83.33	36.46
PA	00.00	14.00	22.33	27.66	51.00	22.99
KA	12.00	17.33	25.33	40.33	66.33	32.26
KP	10.66	16.66	24.00	30.33	53.33	26.99
CHP	12.00	17.33	25.00	39.66	60.00	30.79
KO	11.33	17.00	24.66	39.00	60.00	30.39
MA	11.33	17.00	24.66	39.00	59.66	30.33
MMT	10.66	16.66	24.00	29.66	52.33	26.66
SWP	00.00	17.33	24.00	40.00	60.00	28.26
GA	12.00	17.33	24.33	40.00	60.00	30.73
Mean	9.33	16.86	24.46	36.73	60.59	
	CD (P = 0.05)		CD (P = 0.01)			
Isolates	0.431		0.572			
Days	0.272		0.362			
Isolates×Days	0.862		1.144			

Table 2. Radial growth of 10 isolates of *Catenaria anguillulae* on linseed oil-cake agar medium at 30±1°C

Isolate	Period of observation (Day)				Mean
	Radial growth (mm)				
	3	6	9	12	
VF	22.33	45.00	66.66	83.33	54.33
PA	14.00	27.66	38.33	51.00	32.74
KA	18.33	34.33	55.00	66.33	43.49
KP	15.33	29.66	41.33	53.33	34.91
CHP	16.00	32.00	47.66	60.00	38.91
KO	16.00	31.66	47.33	60.00	38.66
MA	16.00	31.33	47.33	59.66	38.58
MMT	14.00	28.33	40.33	52.33	33.74
SWP	16.00	31.00	47.66	60.00	38.66
GA	16.00	31.33	48.33	60.00	38.91
Mean	16.39	32.23	47.96	60.59	
	CD (P = 0.05)		CD (P = 0.01)		
Isolates	0.431		0.572		
Days	0.272		0.362		
Isolates×Days	0.862		1.144		

isolates were characterized as fast growing (VF isolate), slow growing (PA, MMT and KP isolates) and medium growing (KA, CHP, KO, MA, SWP and GA isolates). The radial growth of VF isolate was significantly higher than other isolates on all days of observations (i.e. 3, 6, 9 and 12 day of incubation). The average rate of growth of this isolate based on observations on 12th day was 6.5 mm per day. However, the maximum rate of growth for this isolate during 4~6 day of incubation was 7.55 mm per day .In contrast, average rate of growth for PA, MMT and KP isolates were 3.83, 3.94 and 4.02 mm per day respectively. The growth rate of isolates with medium growth ranged between 4.55 to 5.11 mm per day.

At 35°C, radial growth (Table 3) of isolates: VF, PA, KA, SWP, GA, CHP, KO and MA showed significant increase as compared to 30°C, which was found to be optimum for KP and MMT isolates. At this temperature maximum radial growth was recorded for VF isolate followed by KA, SWP, CHP, KO, GA and MA isolates with their respective rate of growth i.e. 8.03, 7.25, 6.40, 5.29, 5.18, 5.14 and 5.07. Thirty five °C was found to be optimum for KA, SWP, CHP and KO isolates at which maximum radial growth of these isolates was recorded as compared to other temperatures included in this study.

Observations on radial growth of all the isolates at 40°C (Table 4) clearly indicated that growth of only GA isolate increased as compared to preceding temperatures, while growth of VF isolate was comparable to that of 35°C on third day of inoculation. Two isolates : KP and MMT did not show any growth. However, the other isolates, showed growth, which was significantly lower than the radial growth recorded at 35°C or 30°C. It was interesting to note that GA isolate, which showed medium growth at 30°C, showed significantly marked increase in radial growth/growth per day at 40°C.

When these isolates were grown at 44±1°C, only four isolates VF, KA, SWP and GA showed growth (Table 5). It is worth noting that the maximum radial growth at this temperature was recorded for isolate GA followed by VF, KA and SWP. The other isolates PA, CHP, KO and MA, however, failed to grow at this temperature. The maximum rate of growth (8.0 mm) was recorded for GA isolate followed by VF isolate (7 mm) on second day of incubation. The maximum rate of growth for KA and SWP were 5.0 mm and 3.6 mm per day respectively. It is

Table 3. Radial growth of 10 isolates of *Catenaria anguillulae* on linseed oil-cake agar medium at 35±1°C

Isolate	Period of observation (Day) Radial growth (mm)			Mean
	3	6	9	
VF	25.00	50.67	78.66	51.44
PA	15.00	26.33	41.33	27.55
KA	23.67	44.67	70.33	46.22
KP	14.33	26.33	37.33	25.99
CHP	18.00	35.33	52.67	35.33
KO	17.67	34.33	51.67	34.55
MA	17.00	32.67	49.67	33.11
MMT	13.67	24.67	36.00	24.78
SWP	22.00	40.33	62.67	41.66
GA	18.33	34.33	51.33	34.66
Mean	18.46	34.96	53.16	

	CD (P = 0.05)	CD (P = 0.01)
Isolates	0.505	0.674
Days	0.276	0.369
Isolates×Days	0.875	1.168

Table 4. Radial growth of 10 isolates of *Catenaria anguillulae* on linseed oil-cake agar medium at 40±1°C

Isolate	Period of observation (Day) Radial growth (mm)		Mean
	3	6	
VF	26.33	45.33	35.83
PA	13.00	25.33	19.16
KA	17.66	35.00	26.33
KP	00.00	00.00	00.00
CHP	13.33	24.66	18.99
KO	11.33	22.33	16.83
MA	8.33	14.66	11.49
MMT	00.00	00.00	00.00
SWP	16.33	33.66	24.99
GA	21.33	37.66	29.49
Mean	12.76	23.86	

	CD (P = 0.05)	CD (P = 0.01)
Isolates	0.526	0.702
Days	0.333	0.444
Isolates×Days	1.053	1.405

Table 5. Radial growth of 10 isolates of *Catenaria anguillulae* on linseed oil-cake agar medium at 44±1°C

Isolate	Period of observation (Day) Radial growth (mm)			Mean
	1	2	3	
VF	6.66	13.66	17.66	12.66
PA	00.00	00.00	00.00	00.00
KA	6.33	11.66	16.66	11.66
KP	00.00	00.00	00.00	00.00
CHP	00.00	00.00	00.00	00.00
KO	00.00	00.00	00.00	00.00
MA	00.00	00.00	00.00	00.00
MMT	00.00	00.00	00.00	00.00
SWP	6.66	11.00	14.66	10.77
GA	6.66	14.66	20.33	13.88
Mean	2.63	5.09	6.93	

	CD (P = 0.05)	CD (P = 0.01)
Isolates	0.290	0.387
Days	0.183	0.245
Isolates×Days	0.580	0.775

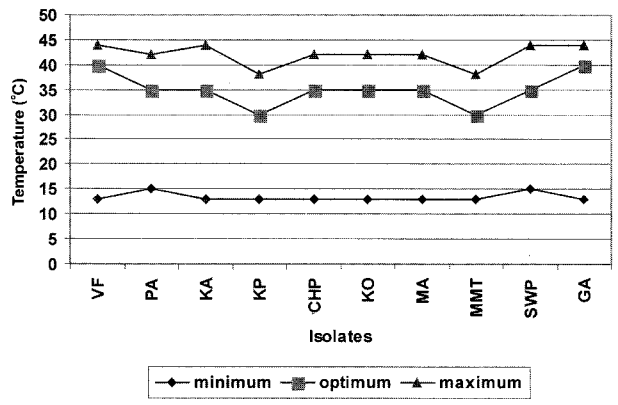

Fig. 1. Minimum, maximum and optimum temperature for the growth of 10 isolates of *Catenaria anguillulae*.

again important to mention that GA isolate which proved to be thermotolerant, showed constantly increase in growth with increase in temperature, while VF isolate started growing at much faster rate at 30°C and above. Thus temperature studies seem to be very important for study of variability in *C. anguillulae*. Growth of VF, GA, KA and SWP isolates was completely checked at 46±1°C.

Cardinal temperatures for the growth of 10 isolates of *C. anguillulae*. Figure 1 shows the cardinal temperatures for the growth of 10 isolates of *C. anguillulae*. The minimum temperature for the growth of 8 isolates VF, KA, KP, CHP, KO, MA, MMT and GA was found to be 13°C, whereas for two isolates, PA and SWP, it was recorded to be 15°C. Similarly, 40°C was found to be optimum temperatures for two isolates (VF and GA), 35°C for six isolates (PA, KA, CHP, KO, MA and SWP) and 30°C for two isolates (KP and MMT). The maximum temperatures for the growth of different isolates also varied. Four isolates : VF, KA, SWP, and GA grew at 44°C, four isolates : PA, CHP, KO and MA grew at 42°C, while two isolates KP and MMT grew at 38°C.

Effect of different temperature on *In vitro* pathogenicity test of VF, KP and MA isolates of *C. anguillulae* against *X. basiri*. The pathogenicity of the three isolates of *C. anguillulae* varied significantly at different temperatures. Maximum percentage of infection of *X. basiri* was observed at 40°C with VF isolate, whereas, 30°C was found to be optimum for isolates KP and MA for their maximum parasitism. VF isolate caused infection at a temperature range of 20~40°C, whereas, KP and MA isolate parasitized nematodes only between 20~35°C (Table 6).

Morphological variation in different isolates of *C. anguillulae* at different temperatures in linseed oil-cake agar medium. The effect of temperature on morphometricl variations of sporangia, discharge tubes and isthmuses are presented in Table 7. The conspicuous effect of temperature on size of sporangia was observed in four isolates : VF, GA, KA and SWP all producing filamentous or elliptical sporangia. In general, the sporangial length decreased at lower and higher temperatures, whereas width of sporangia increased with increase or decrease of temperatures. In VF isolate which produced predominantly filamentous sporangia this effect was most conspicuous. In this isolate, width of sporangia was maximum at 44°C and most of the sporangia were oval-elliptical or spherical (Fig. 2). In GA isolate at 40°C sporangia were smaller and spherical. The sporangia of the two other isolates became almost spherical with abundant rhizoids. More or less in the same manners, the width in CHP and KO isolates also increased with increase or decrease temperature, while length of sporangia decreased with increase or decrease of temperature. The other isolates did not show conspicuous variation. The size of discharge tubes and isthmuses also varied to some extent in relation to temperature.

From the data on the effect of temperatures on radial growth of different isolates, it is clear that isolates of *C. anguillulae* exhibit a high degree of variability with respect to temperatures which perhaps has not been established earlier. The wide distribution of *C. anguillulae* during all the months and seasons in soil (Barron, 1977; Persmark, 1995; Vaish and Singh, 2002) may be attributed, if not wholly, partly to varying optimum temperatures or range of temperatures required for different isolates. Stirling and Platzer (1978) reported growth of an isolate of *C. anguillulae* at 9~42°C, optimum being 33°C. Birchfield (1960a) reported that the fungus grew fast at optimum temperatures of 28~33°C and minimum and maximum temperatures of 9°C and 43°C respectively.

Table 6. Effect of different temperature on *In vitro* pathogenecity test of VF, KP and MA isolates of *Catenaria anguillulae* against *Xiphinema basiri* on 4th day at 7.0 pH

Temperature (°C)	Mortality at different temperature (%)			Mean
	Isolates			
	VF	KP	MA	
15	0.00	0.00	0.00	0.00
20	38.33	20.00	16.66	24.99
25	43.33	33.33	28.33	34.99
30	68.66	56.66	45.00	56.10
35	83.33	13.33	43.33	46.66
40	90.00	0.00	0.00	30.00
Mean	53.60	20.55	22.22	

	CD (P = 0.05)	CD (P = 0.01)
Temperature	4.553	6.120
Isolates	3.220	4.238
Temperature×Isolates	7.886	10.600

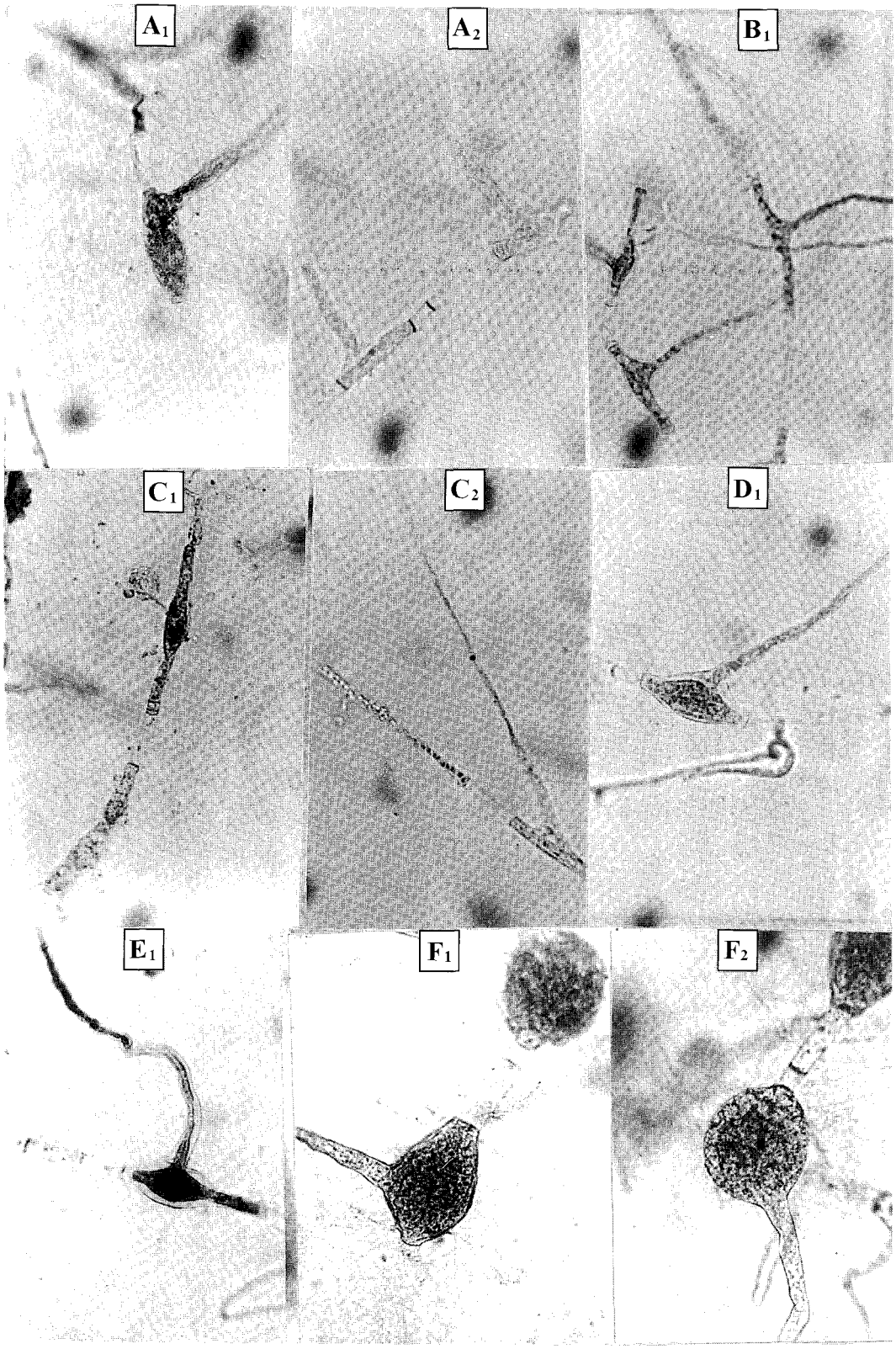

Fig. 2. Sporangia of VF isolate of *Catenaria anguillulae* on linseed oil-cake agar medium at different temperatures (×220). A_1 : 20°C, A_2 : 20°C, B_1 : 25°C, C_1 : 30°C, C_2 : 30°C, D_1 : 35°C, E_1 : 40°C, F_1 : 44°C, F_2 : 44°C.

Stirling and Platzer (1978) also reported that the zoospore production was largely restricted between 21~39°C with an optimum of 27~33°C. However, in VF isolate zoospore production was abundant at 40 and 44°C.

VoB and Wyss (1990) reported that for most isolates of *C. anguillulae*, the optimum temperature for hyphal

Table 7. Size of sporangia, discharge tubes and isthmuses (μm) of different isolates of *Catenaria anguillulae* at different temperature on linseed oil-cake agar medium

Isolate		Temperature (°C)					
		20	25	30	35	40	44
VF	Sporangium	36~91×18~33	39~137×9~24	57~213×6~21	45~108×9~30	48~91×15~45	45~76×33~61
	Discharge tube	121~409×6~9	131~463×4~6	109~530×3	122~549×3~6	67~262×4~6	24~347×6~9
	Isthmus	12~64×6	18~61×6	9~106×6	12~82×6	15~84×6	39~85×6~9
PA	Sporangium	15~30×21~27	15~27×21~30	12~24×15~33	15~24×18~33	15~24×18~33	–
	Discharge tube	30~42×4~6	24~54×4~6	12~64×3	30~88×3~6	30~91×3~6	–
	Isthmus	67~137×6	39~170×6	15~51×6	18~61×6	42~97×6	–
KA	Sporangium	36~97×12~42	36~128×6~35	61~213×6~42	42~97×9~37	30~61×30~42	27~64×24~58
	Discharge tube	207~430×6~12	76~433×6	67~356×3~6	73~494×6~9	353~555×4~9	24~106×6
	Isthmus	15~61×6	15~54×6	9~67×6	24~73×6	6~67×6	12~61×6~9
KP	Sporangium	36~48×36~54	24~45×24~42	27~39×27~33	39~48×42~61	–	–
	Discharge tube	158~335×6~9	42~430×6~9	228~564×3	94~189×9~12	–	–
	Isthmus	6~30×6	12~45×6	15~51×6	6~18×6	–	–
CHP	Sporangium	27~61×24~76	30~61×27~54	36~61×15~24	33~103×15~36	15~30×30~54	–
	Discharge tube	15~64×12~24	18~39×9~15	33~219×3~6	24~524×6~12	6~21×9	–
	Isthmus	15~67×6~9	12~85×6	24~106×6	6~84×6	15~128×6	–
KO	Sporangium	21~45×18~42	21~39×15~27	24~67×15~18	18~39×24~48	15~48×15~45	–
	Discharge tube	9~30×9~18	9~15×6~12	97~372×3	24~439×6	9~36×6~12	–
	Isthmus	21~70×6	15~73×6	15~91×6	12~115×6	18~82×6	–
MA	Sporangium	30~45×24~48	30~45×36~54	30~45×36~48	45~67×57~70	45~64×51~73	–
	Discharge tube	265~55×6~9	36~445×6~15	67~317×3~9	183~359×9~15	167~347×9~15	–
	Isthmus	15~36×6	15~103×6	6~45×6	12~24×6	12~30×6	–
MMT	Sporangium	24~33×24~36	33~45×24~42	27~45×30~45	30~48×48~61	–	–
	Discharge tube	106~173×6~9	97~262×6~12	67~399×3~9	219~417×9~15	–	–
	Isthmus	15~35×6	18~45×6	15~82×6	6~24×6	–	–
SWP	Sporangium	36~70×21~42	36~79×12~34	45~91×15~36	51~97×15~37	36~79×21~45	21~61×27~48
	Discharge tube	125~372×6~9	109~427×6~9	36~280×3	76~356×6	24~420×4~9	30~70×6~9
	Isthmus	15~97×6	24~61×6	6~106×6	21~91×6	15~91×6	24~67×6~9
GA	Sporangium	39~106×15~27	42~13×12~27	54~143×6~18	39~91×12~30	27~76×18~76	24~45×24~33
	Discharge tube	73~292×4~6	128~478×4~9	18~262×3	237~646×3~6	106~292×4~12	24~48×6
	Isthmus	24~67×6	9~~51×6	15~91×6	15~106×6	12~67×6	27~51×6

growth was 30°C (9 out of 16 isolates), others showed increasing growth rates above 30°C (5 out of 16 isolates) indicating a higher temperature tolerance. As a rule the growth of all strains was lowest at 15°C. They also found significant difference between the isolates with maximum growth rates ranging between 2.2 and 5.8 mm per day. From their observations it may be concluded that there was variability in isolates of *C. anguillulae* in relation to temperature.

In the present study out of four isolates VF, GA , KA and SWP, the former two were considered as thermotolerant, while KA and SWP were less thermotolerant because of their optimum temperature, being 40°C and 35°C respectively. Of the two thermotolerant isolates (VF & GA), VF isolate showed abundant zoospore production at 40°C and 44°C. GA isolate, although, grew better than VF isolate at 44°C, it apparently did not produce zoospores. Abundant production of zoospores at higher temperatures indicates that VF isolate may infect nematodes even at 44°C, which may be lethal for most of the nematodes.

Observations on the effect of different temperatures on pathogenicity of *C. anguillulae* on *X. basiri* clearly indicate that optimum temperature as well as the range of temperature for infection varied significantly (Table 6). The optimum temperature for infection of VF isolate was 35~40°C, whereas, 30°C was optimum for KP and MA isolates (Table 6). Higher percentage of infection at wide range of temperature (20 to 40°C) by VF isolate may be attributed to thermotolerant nature of the isolate. This isolate grew best at 35~40°C with good growth even at 44°C and also produced abundant zoospores at these temperatures which may be essentially responsible for increased infection at 40°C. Additionally the nematodes were also immobilized at higher temperature which must have facilitated for rapid infection. In contrast, KP and MA isolates growing best at 30°C, caused maximum infection at 30°C. The growth of these two isolates was almost completely inhibited at 40°C, which may be accounted for escape of infection at this temperature.

The morphology of zoosporangia in isolates particularly the thermotolerant ones varied with increasing temperature also. VF, a thermotolerant isolate, producing fila-

mentous sporangia mostly at 30°C produced only broadly elliptical and spherical sporangia with relatively shorter discharge tubes at higher temperatures. In contrast, GA isolate produced smaller spherical sporangia (Table 7). The change in the morphology of sporangia in response to varying temperatures can not be explained satisfactorily at this stage.

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