

## VI. SUMMARY AND CONCLUSIONS

### 1. Life history of P. hygrophilus

Though Bassen (1940) first cultured Panagrolaimus hygrophilus on nutrient agar with pieces of Neleumbium nucifera, this nematode could be easily cultured in several media.

Eggs deposited by female P. hygrophilus ranged from 6 to 10 with an average of 7.2. The embryonic development was similar to that of several free living nematodes and the duration ranged from 75 hr 5 min to 84 hr 40 min in the fertilized and unfertilized eggs respectively. The duration of development in the juvenile stages was 2, 3, 4 and 4 days respectively and adults oviposited on the 13th day. The total duration from egg to egg stage was 14 to 16 days.

### 2. Morphology

Eggs measured  $45 \pm 0.3$  micra in length and  $23.4 \pm 0.3$  micra in breadth while in uterus of female and after oviposition, the length increased by 0.2 micra. Egg had an outer shell and an inner membrane typical of most free living nematode eggs.

Detailed description of all the juveniles and adults P. hygrophilus were made for the first time. Juveniles resembled adults. Sexual differentiation was obscure in the first stage. The germinal primordium was located at 50.4 per cent of the length from head end in second stage female and at 64 per cent from head end in the second stage male. In the adult female, the gonad was Panagrolaimoid, prodelphic, ovary tip being reflexed past the vulva as far as the anus. Post vulva sac was about  $3/4$  to  $1/3$  anal body width. Vulva was transverse and V was  $55.6 \pm 3.3$  per cent. Absence of bursa and presence of pre-anal lateroventral and terminal pairs of caudal papillae differentiated the males. Spicules were double, arcuate bent with a rounded head followed by a neck and pointed apex. Gubernaculum was 8 micra long.

### 3. Growth of P. hygrophilus and biomass production

The body length of first stage juvenile measured 285 micra which had increased by 1.3, 1.9, 2.1 and 2.9 times in females and by 1.4, 1.6, 1.6 and 1.8 times in the male adults. In general, the female was longer ( $0.921 \pm 0.02$  mm) than the males ( $0.864 \pm 0.02$  mm) and broader width was  $24.3 \pm 0.5$  micra in females and  $20.0 \pm 0.7$  micra in males. The gonad length which was 13 micra in the second stage juvenile had increased by 29.6 times

in female and by 30.7 times in the adult males.

The biomass of juveniles emerging from egg was 0.0277 micrograms which had increased gradually by 11.9 times in adult females and by 7.8 times in males. There was a greater increase in the juveniles developing into adult females and the increase in body length, intestine and maximum body width was substantial from the fourth stage juvenile to the adult.

#### 4. Biochemical constituents of *P. hygrophilus*

Starvation of the nematode, *P. hygrophilus* reduced total protein by 38.7%, amino acids by 10.7% and total sugars by 13.3% due to alteration of the internal body reserves. Among the enzyme systems, catalase increased by 2.64, polyphenol oxidase by 0.32 times, amylase by 2 times, acid phosphatase by 0.189 times and urease by 20%. Peroxidase was reduced by 0.64 times, cellulose by 24.4% perhaps due to lack of nutrition.

#### 5. Pure culture of *P. hygrophilus*

Among the ten culture media tested for suitability to *P. hygrophilus*, only in oat meal agar 4% and malt extract, over 40% of final population became adults. There was a significant increase in the percentage of females (82.6) in oat meal agar indicating its suitability.

A significant increase in body length and width of adult female was observed in oat meal agar 4% and malt extract and the 'a' values were therefore smaller. The visible increase in the diameter at about 10-20 micra towards the head end from the vulval region indicated that a well developed oocyte was positioned in that region and this parameter may vary with the state of development of oocyte in the uterus at the time of measurement. In both the females and males the body length fluctuated by 4-5%, that by 10% and maximum body width by 15%. Though the total body length was higher in PDA than in OMA 4%, the general increase in the female population in OMA 4% was evident.

The average biomass was 1.1341, 4.8735, 8.6281 micrograms in the juvenile stages and 12.9814 micrograms in adult female. In the males, these weights were 0.8315, 1.613, 3.0244 respectively, indicating a three-fold increase in adult females over males which may be due to the development of oocytes. The total biomass of all motile stages was maximum(73.1245) in oat meal agar 4% and hence, this medium was found to be suitable for culturing P. hygrophilus.

#### 6. Effect of chemicals on survival of P. hygrophilus

Exposure of P. hygrophilus to chemicals for assay on osmoregulation showed the  $LC_{50}^p$  values as 86.1, 71.9

and 125.6  $\mu$  for formic acid, butyric acid and acetic acid. For salts, the values were 0.50  $\mu$  sodium phosphate, chloride, and 144.5  $\mu$  for potassium chloride. All salts were tolerated upto 100  $\mu$ . With carbohydrates viz., sucrose, glucose and maltose lower doses of 50 and 100  $\mu$  were tolerated.

7. Morphometrics and biomass production of *P. hygrophilus* in media fortified with chemicals

The total number of inoculations made in the investigation was 294. The treatments sampled for morphometric study and biomass estimation were 2948 and the measurements numbered 8 to 9 for each specimen. In order to reduce the enormous data and to facilitate comparisons, only adults were taken for measurements. Among the parameters, the gonad measurement was restricted to the females only and the results were interpreted.

(a) Among the salts, chloride and nitrate of sodium at 25  $\mu$  and phosphate of sodium at 50 and 100  $\mu$  increased the number of females in the final population. These, and phosphate of potassium at the above doses were effective. The 'b' values increased by 2.1 to 11.5% among salts indicating no additional growth of nematode oesophagus. A positive relationship existed between the body length and gonad length.

In the males, the body length and tail length were within the range of variation and so were the ratios  $a$ ,  $b$ ,  $b^1$  and  $c$ .

(b) Among the amino acids, threonine and glycine at 50 and 100  $\mu$ , serine and glutamic acid at 12  $\mu$  and phenylalanine at 50  $\mu$  showed increase in the total female population and biomass. In these treatments, there was a reduction in 'a' value. Gonad length varied from 370 micra in glycine and alanine 12  $\mu$  to 990 micra in glutamic acid 25  $\mu$ . A positive correlation existed between the body length and gonad length. Threonine, lysine and methionine seemed to be essential for female and serine, glutamic acid and phenyl alanine were needed by males.

(c) Among the vitamins, multi-vitamin, vitamin-C at 50  $\mu$ , becozyme and ravingon at 12 and 100  $\mu$ , dexasol (cold) at 25 and 50  $\mu$ , dexasol (hot) at 25  $\mu$  and adrenalin chloride at 25  $\mu$  increased females and there was a general increase in biomass. The 'a' values were higher in multi-vitamin 50  $\mu$  and dexasol 25  $\mu$  because the body width of female did not increase correspondingly with the body length. A positive correlation existed between body length and gonad length.

(d) Among the growth regulators tested, only cholesterol followed by insulin showed higher percentage of females in the final population, but the biomass increased by 9.2% in the former and decreased by 3.5% in the latter. The body length of the female was reduced by 10% in insulin 6000 units. The gonad length ranged from 320 micra in thiourea 12 m $\mu$  to 398 micra in cholesterol 12 m $\mu$ . There was a reduction in insulin 6000 units and in thiourea 25 m $\mu$  and SR 25 m $\mu$  indicating the inhibitory effects of the growth promoters on P. hygrophilus. The successful growth of nematode and increase in biomass in cholesterol confirmed the need for sterol as an ingredient in the media for culture of P. hygrophilus.