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## POSTURE CHANGES IN *HELICOTYLENCHUS INDICUS* AS INFLUENCED BY COPPER SULPHATE AND TEMPERATURE

by

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Nematodes upon relaxation assume a posture which is characteristic and thus may be useful in their identification. The plant parasitic nematodes of the genera Rotylenchus Filipjev, 1936 and Helicotylenchus Steiner, 1945 are typical examples of posture characteristics, as they assume a spiral posture upon death or on relaxation with gentle heating. Helicotylenchus indicus Siddiqi, 1963 assumes a characteristic flat spiral posture when relaxed. Any change in the media (environment) in which they live disturbs their normal spiral posture. The posture of nematodes is influenced by physical and chemical stimuli due to abnormal neuro-muscular activity and hence such changes provide a useful tool for the study of nematode behaviour. Recently Keeth (1974) has studied the effect of aldicarb on the posture of Aphelenchus avenae. Jairajpuri et al. (1975) have studied the effect of copper sulphate on some nematodes and came to the conclusion that lower concentrations (0.025 M) of this salt is not very toxic to H. indicus. The postural patterns of nematodes show its activity viz., resting or locomotory state. A sudden change in the temperature produces marked changes in their posture. The present paper deals with the changes that occur in the posture of H. indicus when treated with copper sulphate solution, and also in response to very high or very low temperatures.

## MATERIALS AND METHODS

The nematodes were collected from the University Campus, Aligarh and extracted by modified Baermann's funnel technique. They were placed in batches of 20 in petri-dishes containing tap water, distilled water, or 2% copper sulphate solution (2g/lit). A 2% concentration of copper sulphate solution was used because it is not toxic to the nematodes but still causes significant changes in posture. The postures which *H. indicus* assumes in each of the above media were drawn with the help of a camera lucida after intervals of 30 and 60 minutes. The analysis (Fig. 1) of different postures assumed by the nematodes in each media was done using the following measurements: L = length of the animal; l = length of the spiral along the axis; X = distance between head and tail; V and D = distance between the lines drawn parallel to the axis of spiral.

From the values obtained the following ratios were calculated: A = L/l; B = 1/X; C = V or D maximum/V or D minimum.

In copper sulphate solution and tap and distilled water, six

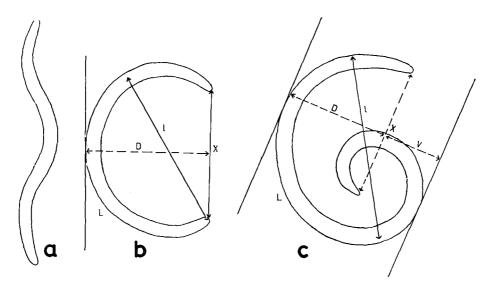


Fig. 1 - The postures of *Helicotylenchus indicus* and the values that were measured: a - wavy posture showing locomotory phase; b - C-shaped posture; c - spiral posture showing the resting phase of the animal. L - length of the animal; 1 - length of the spiral along the axis; X - distance between the head and tail; V and D - distance between the lines drawn parallel to the axis of the spirals.

main postures of *H. indicus* were recognized. The frequency of occurrence of each was estimated, and the subsequent changes per minute which took place in tap water and in 2% copper sulphate solution.

The effects of changes in the temperature on the posture changes were studied by placing 50 nematodes in each of 8 petri-dishes containing 5 ml of water kept at room temperature  $(28\pm2^{\circ} \text{ C})$ . The number of nematodes in spiral, intermediate or wavy postures were noted at intervals of 1, 2 and 4 hours. The petri-dishes were then kept at 8° C for 6 hours and afterwards again kept at room temperature for the purpose of making observations as the temperature gradually increased from 8-28° C.

# **RESULTS AND DISCUSSION**

During locomotion *H. indicus* assumes a wavy posture and its ventral body surface faces substratum, but in the resting state one of the lateral surfaces is towards the substratum (Fig. 1). It was also found that the animal is in simple wavy posture during locomotory state, whereas when stationary it assumes a flat spiral posture. The other postures represent transitionary phases of the nematodes between the stationary and locomotory states.

The lower values of 'a' show that the axial length of the spiral is high which is correlated with a greater relaxation of body muscles (open spiral). The higher values of 'B' show that the head and tail are closer to each other. The value of 'C' shows degree of coiling and spiralling of the body. An infinite value indicates a C-shaped posture of the nematode, while unit indicates a closed spiral posture.

As evident from Fig. 2, relaxation was maximum in distilled water after 30 minutes treatment, whereas in copper sulphate solution relaxation and contraction were generally of equal frequency. However, in tap water only slight relaxation was observed. After 60 minutes of treatment those specimens in copper sulphate solution showed maximum relaxation, while minimum relaxation was observed in distilled water and normal relaxation and contraction occurred in tap water.

Fig. 3 gives the values of 'B' which shows the tendency of the nematodes to close their anterior and posterior extremities in adverse media. After keeping for 30 minutes in distilled water and copper sulphate solution, the two extremities of nematodes came

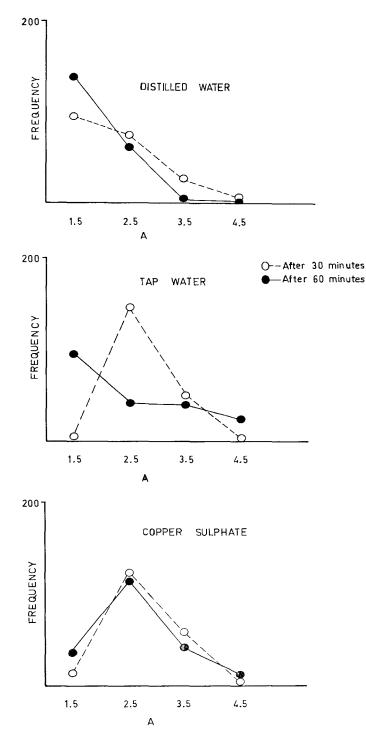


Fig. 2 - The frequency distribution polygon showing the distribution of value 'A' (L/l) in distilled water, tap-water and in 2% copper sulphate solution at intervals of 30 and 60 minutes. Changes in the value of 'A' are significant (p = 0.01).

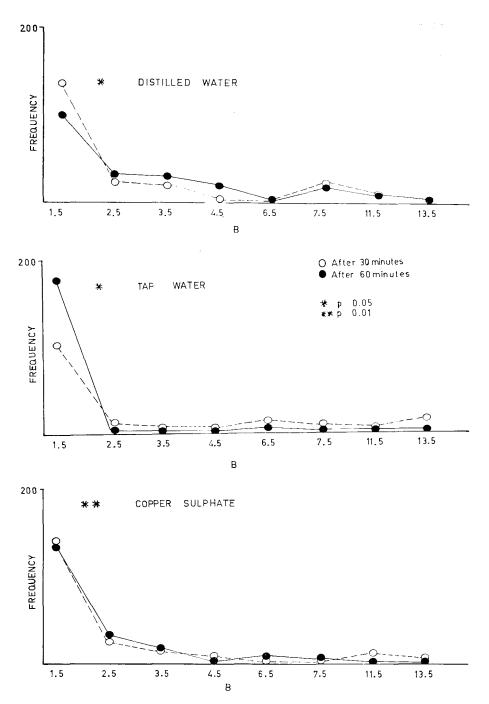


Fig. 3 - The frequency distribution polygon of the value 'B' (1/X) in distilled water, tap-water and in 2% copper sulphate solution at intervals of 30 and 60 minutes.

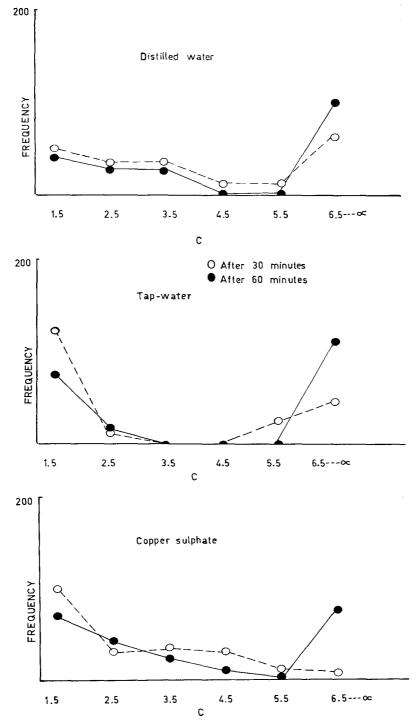


Fig. 4 - Polygon showing the frequency distribution of value 'C' (V or D maximum/V or D minimum) in distilled water, tap-water and in 2% copper sulphate solution at intervals of 30 and 60 minutes. Changes in the values of 'C' are significant (p = 0.01).

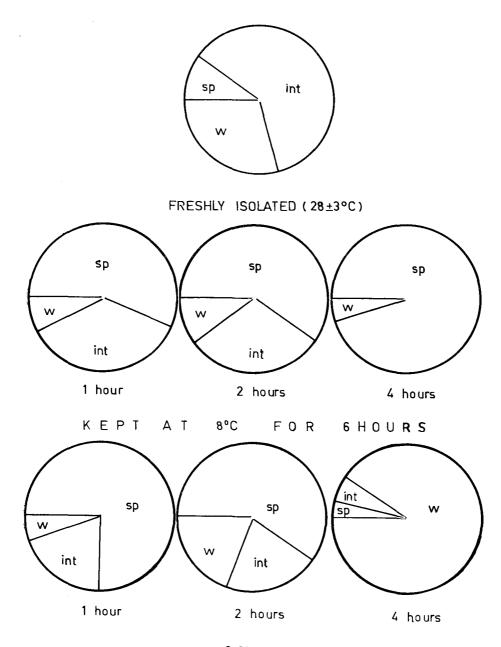
closer, but further closing was seen only in copper sulphate solution after 60 minutes treatment. The rate of closure and withdrawal of the two extremities increased with time in the copper sulphate solution. In tap water only a slight change occurred in posture.

From Fig. 4 the infinite value of 'C' shows C-shaped postures which were observed in tap and distilled waters after 30 minutes, but in copper sulphate solution only after 60 minutes. The spiralling was maximum in copper sulphate, decreasing with time. The unit value (1) of 'C' indicates a typical flat spiral, but fluctuations from this posture occurred more frequently in copper sulphate solution than in water. It also decreased with time.

In table I, six main types of postures are given with their percentages in water and copper sulphate solution. The simple wavy postures were more frequent in tap water after 15 minutes treatment than in copper sulphate solution. In tap water no remarkable change was observed with time. However, when kept in copper sulphate solution for this duration the percentage of wavy posture was less, decreasing gradually up to 30 minutes. The double spirals were more frequent in copper sulphate solution after 15 minutes, decreasing gradually with time. The complex postures, double coiled, and reverse S-shaped postures were more common in copper sulphate solution than in tap-water. The rate of change in posture was higher in tap water.

Fig. 5 shows the different postures assumed by *H. indicus* under the influence of temperature. Nematodes obtained immediately from the extraction funnel showed a lesser percentage of spiral postures, and a higher percentage of intermediate postures. The number of animals in spiral posture increased gradually with time which may have been due to a gradual rise in the temperature of water. It clearly indicates that at high temperatures the nematodes become relaxed by assuming their resting state (spiral postures). When kept at 8° C for 6 hours and then again at room temperature ( $28\pm2^{\circ}$  C) a gradual reduction in the spiral postures was noticed which indicates that the thermal gradient of 8° C to 28° C stimulates the nematodes to assume a locomotory state.

The change in environment (media) is responsible for producing changes in the posture of H. *indicus*. Wavy posture represents locomotory phase of the animal whereas spirals are stationary phase, with the other postures being intermediate stages between the two phases. Treatment with copper sulphate produces a number of changes



p< 0.01

Fig. 5 - Pi - Chart showing the percentage of different postures of *H. indicus* when kept at room temperature after having kept them in fridge: sp - spiral posture; int - intermediate postures; and w - wavy posture. The significance of changes in posture is p < 0.01.

# TABLE I

Percentage of different types of postures observed (Mean of 200 observations)

	S_shaped	spiral		Complex ©	Double coiled
29%	9% (Nine ch	27 % anges pe	20% er min )	7%	8%
9%		•		29%	14 %
3%	17%	10%	18 %	37%	15%
0	0	37%	17%	29%	17%
	>>> 29% 9% 3%	<ul> <li>S-shaped S≥S</li> <li>29% 9% (Nine ch</li> <li>9% 16% (Six cha</li> <li>3% 17% (Five cha</li> <li>0 0</li> </ul>	S-shaped spiral SSS G 29% 9% 27% (Nine changes per 9% 16% 18% (Six changes per 3% 17% 10% (Five changes per 0 0 37%	S-shaped spiral spiral SSS $G$ $G$ 29% $9\%$ 27% 20% (Nine changes per min ) 9% $16\%$ $18\%$ 20% (Six changes per min ) 3% $17\%$ $10\%$ $18\%$ (Five changes per min )	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

Changes in the percentage of different postures are significant (p = 0.01)

in the typical spiral postures of the nematodes. When kept in this media for longer durations tetanic conditions may result. Fluctuations in temperature resulted in postural changes of *H. indicus*.

## ACKNOWLEDGEMENTS

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#### SUMMARY

The effects of changes in media and temperature gradients on postural attitudes were studied in *Helicotylenchus indicus* Siddiqi, 1963. Copper sulphate induced more curling and abnormal postures due to irregular muscular activity. Temperature also caused changes in the postures of the nematode.

### RIASSUNTO

Effetto del solfato di rame e della temperatura sull'habitus di Helicotylenchus indicus.

E stato studiato l'effetto di mezzo e temperatura sui cambiamenti di *habitus* in seno alla specie *Helicotylenchus indicus* Siddiqi, 1963. Ambedue le componenti provocano posizioni innaturali ed avvolgimento su se stesso del corpo del nematode.

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