

MATERIALS & METHODS

Soil sampling : Soil samples from around roots of various field crops and forest trees were collected from various states of India. The samples were taken from a depth of 10-25 cm and were kept in polythene bags. All relevant information such as host, locality, date of collection, etc. were noted. The samples were brought to the laboratory for further processing.

Processing of soil samples: The samples were processed by Cobb's (1918) sieving and decantation technique. About 500 ml soil was placed in a bucket and thoroughly mixed with a small amount of water. The debris and stones were removed and soil lumps, if present, were broken by hand. The bucket was then filled with water to about 3 / 4th of its volume and then the suspension was stirred to make it homogeneous. The bucket was left undisturbed for about 1 / 2 a minute to allow the heavy soil particles to settle at the bottom. The muddy suspension was then poured into another bucket through a coarse sieve (2mm pore size) which retained debris, roots and leaves. The suspension in the second bucket was then poured through a 300 mesh sieve (pore size 53 μ m) . The nematodes and fine soil particles were retained on this sieve. The process was repeated thrice for better recovery of nematodes.

Isolation : The residue on the sieve was collected into a beaker and poured on a small coarse sieve lined with tissue paper. The sieve was then placed on a Baermann's funnel containing water sufficient to touch the bottom of the sieve. Special care was taken to avoid trapping air bubbles between the bottom of sieve and water level. The stem of

the funnel was fitted with a rubber tubing provided with a stopper. The nematodes migrated from the sieve into the clear water of the funnel and settled at the bottom. After about 24 hours a small amount of water was drawn from the funnel through the rubber tubing into a cavity block. The nematodes isolated as above were fixed and processed for mounting on slides.

Killing and fixation: The nematodes collected in cavity block were left undisturbed for a few minutes so as to allow them to settle. Excess of water was removed with a fine dropper and hot TAF (Courtney, Polley & Miller, 1955) was poured into the cavity block. This simultaneously killed and fixed the nematodes.

Mounting and sealing : 36 hours after fixation the nematodes were transferred to a mixture of glycerine-alcohol (95 parts 30% alcohol + 5 parts glycerine) in a small cavity block which was kept in a desiccator containing anhydrous calcium chloride. In about 2-3 weeks the nematodes were dehydrated and ready to be mounted. A drop of anhydrous glycerine was placed on a glass or metallic slide and the nematodes were transferred from the cavity block to this drop. Three pieces of glass wool of same thickness were placed around them to prevent flattening. A cover slip was then gently placed on the drop. The edges of the cover-slip were sealed with nail polish or glyceel.

Measurements and drawings : All measurements were made on specimens mounted in dehydrated glycerine with the ocular micrometer. De Mans (1884) formula was used to denote the

dimensions of the nematodes. All diagrams were drawn using a drawing tube.

Scanning electron microscopy: Freshly isolated nematodes were fixed in 30% gluteraldehyde for 90 min, washed in 0.05 m sodium phosphate buffer several times then post fixed in osmium tetroxide for 2 hours at room temperature and washed again repeatedly in buffer. The specimens were then dehydrated in a graded alcohol or acetone series and critical point dried using carbon dioxide as the transitional fluid. Dried specimens were mounted on stubs using a double side adhesive tape.

Some of the glycerin dehydrated specimens were processed for scanning electron microscopy by the method of Sher & Bell (1975). Glycerine adhering on the surface of specimens was removed by gently touching with filter paper. The dried nematodes were mounted on the edge of a glass chip, which was lightly smeared with glue to hold the nematodes. The glass chips were then fixed to adhesive tape on a stub in an inclined position.

Stubs were coated with 20-30 nm gold in an EIKO III Ion coater and examined in a Hitachi S-2300 scanning electron microscope at an accelerating voltage of 5-15 kv.

Type material : All type material has been labelled and deposited in the Department of Zoology, Aligarh Muslim University, Aligarh. Some paratypes will be deposited in other nematode collection of the world at the time of publication of the description of species.

Abbreviations used in the text:

L = Total body length

a = Body length/greatest body width

b = Body length/distance from anterior end to the pharyngeal base.

c = Body length/tail length.

c' = Tail length/body diameter at anus or cloaca.

V = Distance of vulva from anterior end x 100/body length.

G1 = Distance of the vulva from anterior ovary x 100/body length.

G2 = Distance of the vulva from posterior ovary x 100/body length.

DO = Orifice of dorsal gland.

DN = Nuclues of dorsal gland.

DO-DN = Distance of dorsal gland nucleus from the orific of dorsal gland.

S₁ N₁ = Nucleus of the first ventrosub-lateral gland of the first pair.

S₁ N₂ = Nucleus of the ~~second~~ ventrosub-lateral gland of the first pair.

S₂ N = Nucleus of the ventrosub-lateral glands of the second pair.

S₂ O = Orifices of the ventrosub-lateral glands of the second pair.