

Table 1. Precision and accuracy of modified method in recovering added Br⁻ with bromide ion electrode

Bromide added ($\mu\text{g g}^{-1}$) [*]	Bromide recovery ($\mu\text{g g}^{-1}$) [*]							
	Alfisol				Vertisol			
	Range	Mean	SE	CV (%)	Range	Mean	SE	CV (%)
10	9.5–10.0	9.77	±0.09	2.21	9.6–10.6	9.97	±0.13	3.19
100	96.4–100.0	97.83	±0.62	1.55	97.5–102.5	99.83	±0.67	1.65

^{*}Results based on six determinations

if present in traces in the filtrate can considerably reduce the sensitivity of the ion-selective electrode¹⁴. This analytical procedure was also found to be highly stable and precise (Table 1). Hence, it can be used more successfully for studying the movement of NO₃⁻ in these soils.

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Surface ultrastructure of *Beauveria bassiana* infecting silkworm *Bombyx mori* Linn.

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The surface ultrastructure investigations on entomopathogenic fungi, *Beauveria bassiana* infecting silkworm *Bombyx mori* Linn. reveal that the infecting stage, i.e. oval or spherical conidia are formed on host integument from aerial hyphae. The vegetative hyphae form a network inside the integument and further divide in haemolymph. The crystals of varying size, formed of ammonium and magnesium oxalate have also been observed on integument and in haemolymph.

THE disease, white muscardine caused by entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin in silkworm (*Bombyx mori* L.) has been responsible for considerable silkworm crop loss in the recent past.

The disease is contagious in silkworm and infects the integument, digestive tract, and haemolymph^{1–6}. The life cycle^{3,4} and histological observations on oral infection^{5,6} of *B. bassiana* infecting *B. mori* have been studied earlier. However, no attention has been paid so far on surface ultrastructure study on *B. bassiana* infecting *B. mori* in order to generate further information. Therefore, in the present paper, SEM has been used as a tool to investigate the different stages of life cycle and route of infection of *B. bassiana* infecting *B. mori* to confirm the findings generated by earlier workers based on visual and light microscopy observations.

Third instar larvae of *B. mori* (NB₁₈) were surface infected with 4×10^5 spore/ml and reared on mulberry leaves at $25 \pm 1^\circ\text{C}$ temperature and 60–70% RH. On the seventh day of post infection, larvae were dissected to process the infected integument, digestive tract and trachea. The tissue was fixed in 2.5% glutaraldehyde prepared in cacodylate buffer for 2 h, dehydrated in ethanol series, critically dried, coated with gold, mounted onto copper stubs and scanned under JEOL 100 CX II at 20 kV. Further, a few critically dried samples were also randomly fractured to observe under electron micro-

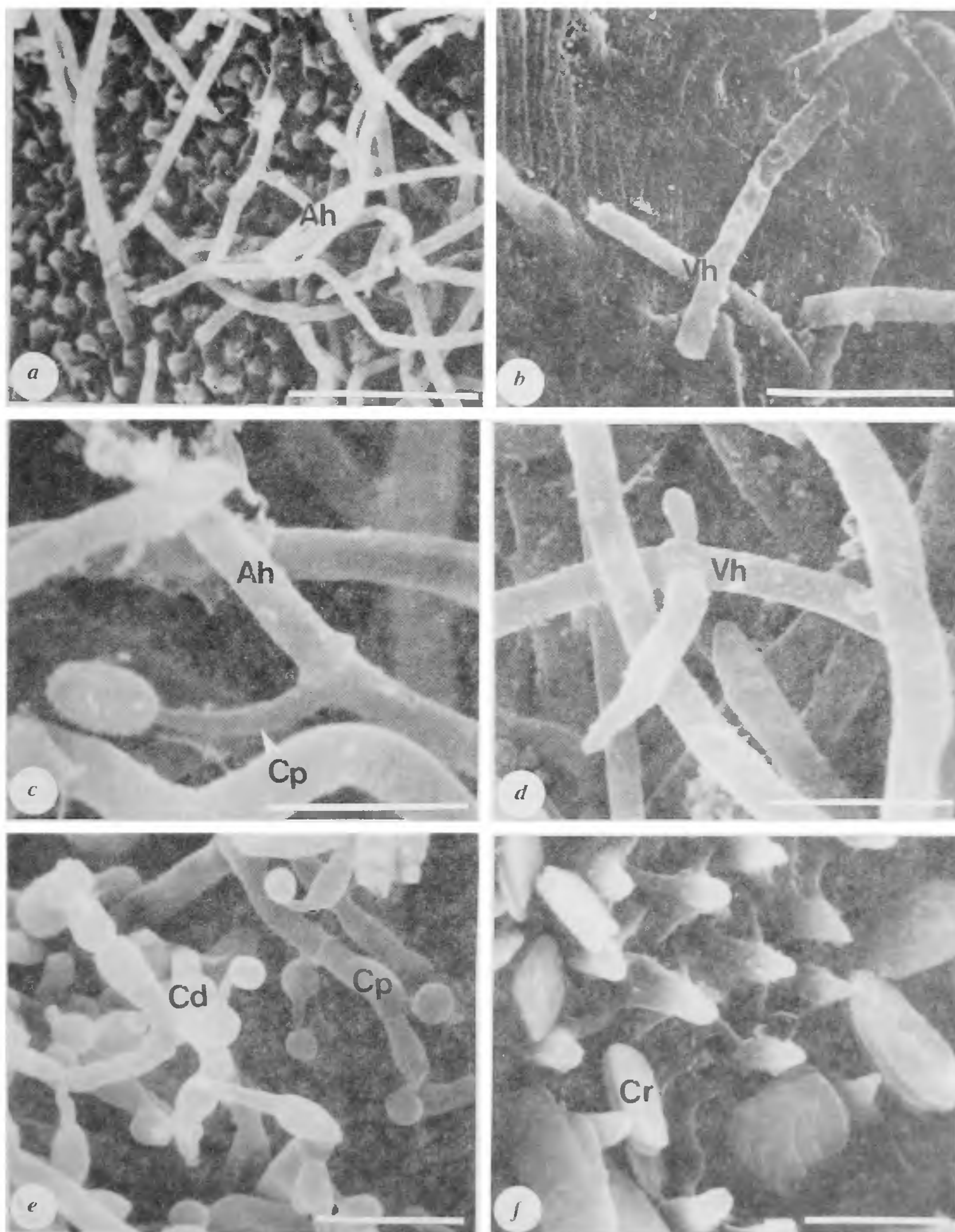


Figure 1 a-f. SEM photographs of *Beauveria bassiana* infecting the silkworm *Bombyx mori* Linn. **a**, Network of aerial hyphae (Ah) over the integument (bar = 10 µm); **b**, Fractured portion of integument showing vegetative hyphae (Vh) inside (bar = 5 µm); **c**, Aerial hyphae (Ah) bearing conidiophores (Cp) (bar = 2 µm); **d**, Dividing vegetative hyphae (Vh) inside the integument (bar = 2.5 µm); **e**, Oval or spherical conidia (Cd) showing detachment. Conidiophores (Cp) can be seen on integument (bar = 3 µm); **f**, Multi-layered ammonium and magnesium oxalate crystals (Cr) deposited over integument (bar = 3 µm)

scope to trace the routes of infection.

The developmental stages of *B. bassiana* are the conidium, vegetative and aerial hyphae. The conidia are oval or spherical shaped (Figure 1 a, c, e). Each conidium germinates within 8–10 h upon contamination of host integument⁴. The conidia put forth a germ tube which releases chitinase, facilitating penetration of the host integument and haemolymph^{1,4}, succeeded by development of several small vegetative hyphae (Figure 1 b, d) which depletes the nutrient and water content of silkworm. The vegetative hyphae also produce toxins causing intoxication and death⁴. After the seventh day of inoculation a large number of vegetative hyphae emerge from the integument to form aerial hyphae, developing into several conidiophores, which give rise to small branches, each of which bear one or more oval or spherical conidia (Figure 1 c, e) similar to the earlier reports^{3,4}. The conidiophores emerge from the base of septum of aerial hyphae (Figure 1 c, e). Each conidium subsequently detaches (Figure 1 e) by osmotic pressure. A large number of conidia on host integument gives a whitish appearance. The multi-layered crystals of ammonium and magnesium oxalate have been observed on the integument of the infected larvae (Figure 1 f) and in haemolymph. The crystals are of different sizes and shapes and considered to be the byproducts of infection. However, the formation and function of

such crystals are not known. The severely infected larva becomes stiff and whitish in appearance because of heavy deposition of conidia and crystals. Generally the infection begins soon after the silkworm integument becomes contaminated with mature conidia. The route of infection through integument in silkworm has been reported by earlier workers based on visual and light microscope observations^{1,2,4}, whereas in the present study we have observed the route of infection under scanning electron microscope which has given a firm support to the earlier reports. Moreover, the oral inoculation of conidia has also been reported causing infection^{5,6}, and sometime infection may also occur through tracheal openings and digestive tract in *B. mori*¹.

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Analysis of trace elements of some edible trematodes parasitizing the bovine hosts

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With the aid of atomic absorption spectrophotometer a qualitative and quantitative analysis of trace element composition of some edible trematode parasites namely, *Gastrothylax crumenifer*, *Fischoederius elongatus*, *F. cobboldi*, *Calicophoron calicophorum*, *Orthocoelium orthocoelium* and *Paramphistomum epiclitum* revealed the occurrence of Cu, Ca, Mg, Mn, Pb, Fe, Ni, Zn, Cr, Cd, K, Se and Co in all the studied species, with K showing the highest concentration and Co the lowest in dry weight of the flukes. Further, Ca, Fe, Zn, Cr and Se were found to be higher in immature and Cu, Mg, Mn, Cd, K and Co were more in mature *G. crumenifer* and *F. elongatus*.

TRACE elements, which are not synthesized in the animal tissue but have significant role in the normal functioning

of the body, constitute an important diet among vital foods. However, both excess and deficiency of any one of these metals may lead to toxicity and metabolic, reproductive and skeletal disorders in the body¹.

Among helminth parasites, the paramphistomid flukes recovered commonly and in abundance from the rumen of cattle and buffaloes constitute an unusual food item and a non-traditional source of animal protein relished by the local tribal population of Meghalaya. In context of helminth parasites, trace elements of several cestode and nematode species have been investigated^{2–5} and their content found to be species-specific⁶. However, similar information with regard to trematode parasites is relatively scanty^{7,8}. The present communication deals with a qualitative and quantitative analysis of trace elements of the edible trematodes all of which are amphistomid digenea.

Live parasites, namely *Gastrothylax crumenifer*, *Fischoederius elongatus*, *F. cobboldi*, *Calicophoron calicophorum*, *Orthocoelium orthocoelium* and *Paramphistomum epiclitum* were recovered from the rumen of cattle, *Bos indicus*, slaughtered at local abattoirs. The mature specimens of all the six species and also the immature (i.e.