SAFFRON STAIN FOR DIFFERENTIATING LIVE AND DEAD NEMATODES

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Summary. Aqueous alkaline and alcoholic extracts of stigmas of flowers of saffron (*Crocus sativus*) at 0.1-1% w/v stained dead nematodes to bright orange or yellowish colour at room temperature within one to two hrs exposure depending on the concentration of the extract, but live nematodes were unstained. Nematodes killed by heat or different chemicals were similarly stained and were not destained when transferred to tap water for several hours, thus allowing sufficient time for observation. Live nematodes were neither killed nor inactivated. The saffron alcohol extract gave the better result. The colour contrast was enhanced when viewed in fluorescent white transmitted light.

Very often difficulty is encountered in differentiating live from dead nematodes. Nematode mortality is often based on their lack of activity (Moje, 1959; Rich *et al.*, 1977), but sometime nematodes become inactive and cease locomotion under the influence of sub-lethal doses of toxic or anaesthetic substances. Therefore, the discrete recording of data base merely on their movement might be subject to errors. Besides, it is time consuming and cumbersome.

The stigmas of saffron, *Crocus sativus* L., plants are used for colouring food and confectionery and seem to differentially stain living and dead nematodes. The contents of this yellow dye from the stigma of the flower are safranal, crocetin, picrocrocetin and crocin (Escribano *et al.*, 1996). Experiments were undertaken to evaluate the efficiency of this stain.

MATERIAL AND METHODS

One hundred mg saffron stigma was soaked in 10 ml glass distilled water for 12 hrs and the filtrate was used as a stain. The stain was stored at 2 °C in a refrigerator. About fifty nematodes killed by heat or chemical, separately, and fifty by natural death were used in the experiment. These were kept in 2 ml of 5% solution in a cavity block and in another cavity block fifty live nematodes were kept at room temperature, 30 °C.

RESULTS AND DISCUSSION

After two hrs it was observed that dead nematodes had taken up the stain while the live nematodes in the active or inactive state were unstained, depending upon the concentration of the extract (Fig. 1). As the exposure time increased the dead nematodes took on a brighter yellow orange colour while live nematodes re-

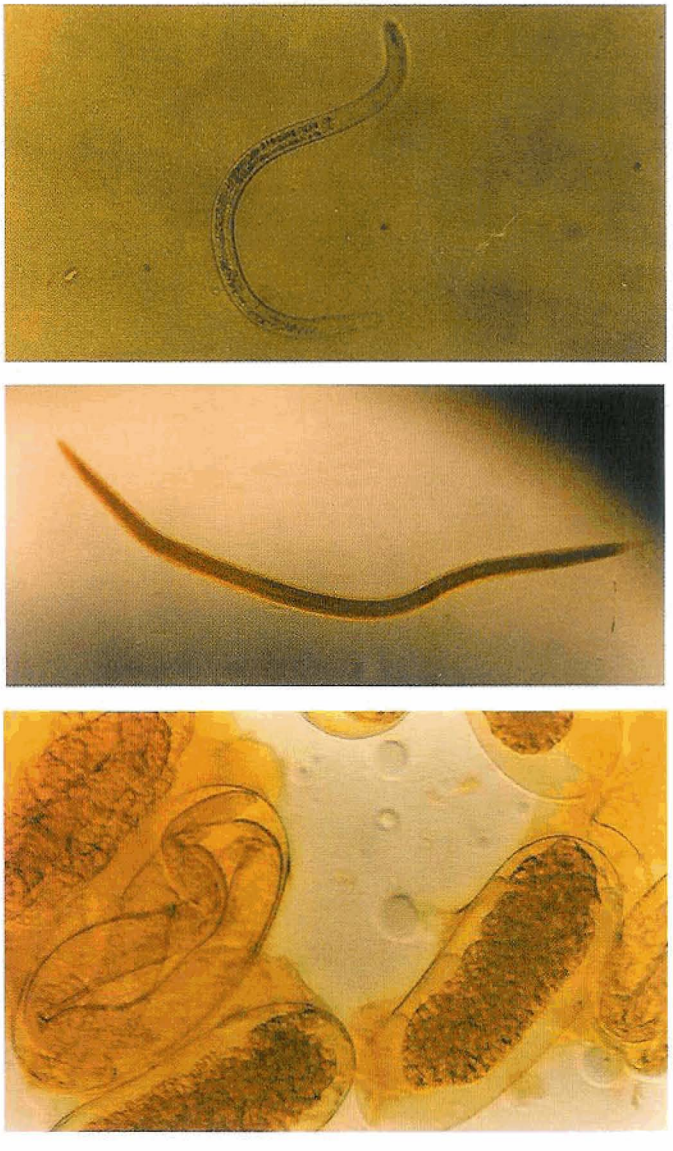

Fig. 1. Effect of saffron on the stain of nematodes: top, active unstained *Pratylenchus*; middle, dead stained *Meloidogyne*; bottom, egg shells not deeply stained compared to egg content (embryo).

mained unstained. When observed in fluorescent white transmitted light under a stereoscopic binocular microscope at x100 magnification, the colour was much brighter. It is not a permanent stain and destaining took place within 3-4 hrs in water. The live nematodes were neither killed nor became inactive in this stain.

Accepted for publication on 5 April 2002.

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