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Short Communication

A simple technique for preservation of female perineal pattern of *Meloidogyne* spp.

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Morphology of female perineal pattern (area around anus and vulva) of the root knot nematode (*Meloidogyne* spp.) is studied to identify the species under the genus as this was originally proposed by Chitwood (2). Several attempts (7,8,6,4,3) have been made to improve the technique for preparation and photographing of perineal patterns of *Meloidogyne* spp. Most of the cases cuticular patterns are photographed to record the morphological characters therein and stored for a short period and later disposed off. However, preservation of the patterns for subsequent study is much not known to those working with routine identification of *Meloidogyne* spp. In fact, the patterns on the *Meloidogyne* spp. female perineum disappear with the time due to dehydration and decaying of adhering body tissues. This study revealed that the patterns prepared from root material processed by NaOCl–Acid Fuchsin method (1) are good enough for preservation of patterns on anhydrous glycerine mounted glass slides. There are good numbers of glass slides of perineal patterns prepared for identification of root knot nematodes from West Bengal, India during 2002. Mature female specimens dissected from galled root tissue stained by NaOCl–Acid Fuchsin and later stored in acidified glycerol. The full grown females were placed in a drop of clear glycerol on glass slide and cut into two halves with the help of modified razor blade to remove the tissues adhered to the portion. The posterior half retaining perineal cuticular pattern of 3-5 specimens is then placed into a drop of 45% lactic acid on the one onside of the same slide for five minutes for easy clearing of body tissues. Fine tip of peacock feather designed for the purpose was used to remove the debris from the inner side of the pattern. The female cuticle is finally trimmed in the glycerol medium to a square shape containing the given perineal pattern. Each pattern was then mounted in anhydrous glycerine on glass slide, covered with round cover slip and sealed with nail polish. More than 100 patterns were kept flat on an aluminium flat-tray of 20 slide capacity and stored in slide cabinet of 2000 capacity at room temperature of 24°C to 30°C for storage. Almost after 10 years, the mounted patterns were examined for stability of the patterns. Interestingly nearly 60% slides were in good conditions with a little growth of fungal colony inside the cover glass. Therefore, remounting of the cuticle was done on anhydrous glycerol. However, the cuticular patterns look clear and intact with greater clarity as compared to photographs taken earlier on the fresh patterns. This observation reveals

that the perineal patterns prepared for routine identification could be preserved for a very long time. Further, the preserved patterns showed greater clarity on the lines and, therefore, could be helpful for undertaking detailed study of the cuticular patterns of the perineum for identification. Thus glycerol mounted female perineal cuticle on glass slide is no doubt useful for storage and future comparison of *Meloidogyne* spp. and the growth of fungal colony under the cover slip may be prevented by using 0.1% $HgCl_2$ or other suitable fungicides.

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