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# EVALUATION OF SOME LOCALLY AVAILABLE INKS IN NEPAL FOR STAINING ARBUSCULAR MYCORRHIZA

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## ABSTRACT
Two inks locally available in Nepal (Chelpark permanent black and Chelpark washable royal blue) were tested for staining arbuscular mycorrhiza. Both the inks stained all structures (arbuscules, vesicles and internal hyphae) successfully. The tested inks are economically feasible and thus may encourage research activities related to arbuscular mycorrhiza in Nepal.

**Keywords:** Arbuscular mycorrhiza, Chelpark permanent black, Chelpark washable royal blue, staining.

## INTRODUCTION
Safer methods of staining arbuscular mycorrhiza in roots have been proposed by different authors [1, 2] as an alternative to the standard staining method [3]. Such alternative methods employ writing inks and vinegar [1] or Faber Castell stamp pad ink and water [2] for staining mycorrhizal roots. As the inks mentioned in the methods are unavailable in developing country like Nepal, the present investigation was undertaken for assessing some locally available inks to stain arbuscular mycorrhiza.

## MATERIALS AND METHODS
### Collecting roots
Two plant species viz; *Parthenium hysterophorus* and *Axonopus compressus* were selected for this study based on mycorrhizal affinity [4, 5]. Twenty plants of each species at the peak of their flowering period were randomly sampled by making 20cm diameter x 20cm deep core, sealed in polythene bags and taken to laboratory. Plant samples of *P. hysterophorus* were collected from vicinity of crop land, whereas *A. compressus* were collected form garden.

### Clearing of roots
Collected root samples were thoroughly washed in running tap water and stored in formyl acetic alcohol. Roots from each sample were cut into 1 cm segments, dispersed in beaker containing water, and six subsamples randomly selected. Subsamples were cleared in 10% KOH [3] at boiling temperature in water bath prior to staining with different inks. Roots were constantly monitored during the clearing process for determining the optimum timing for root clearing.

### Ink staining
Following inks were tested: Chelpark permanent black and Chelpark washable royal blue (Chelpark Company Pvt. Ltd, Bangalore, India). Three subsamples from each plant were stained with each of the selected inks. After clearing, subsamples were rinsed with vinegar and directly stained by boiling in Chelpark permanent black (5%)-vinegar and Chelpark

washable royal blue (5%)-vinegar solutions respectively for 15 minutes [1]. Roots were then destained in lactoglycerol.

## RESULTS AND DISCUSSION

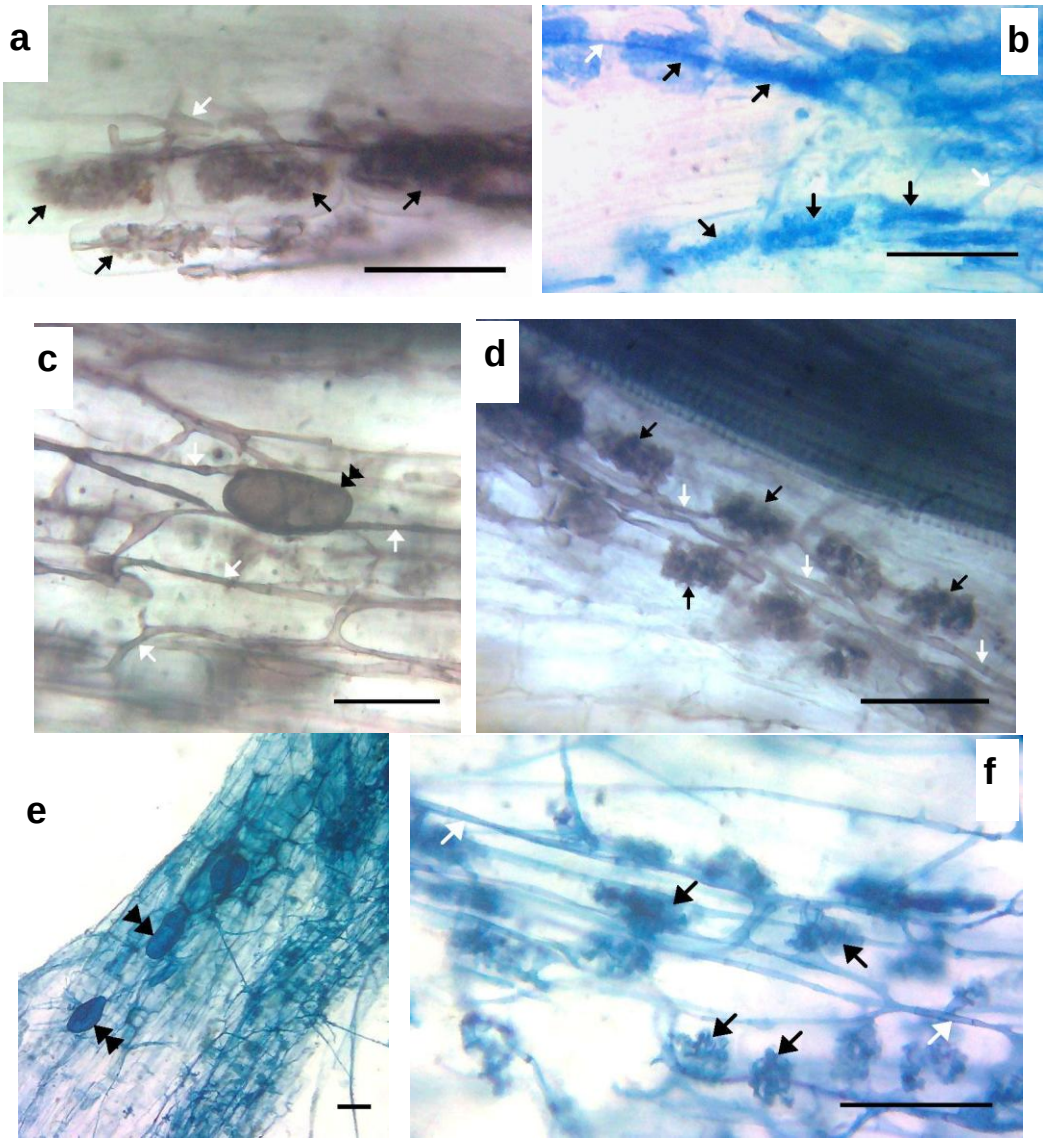


Figure 1 Arbuscular mycorrhizal colonization of roots of (a) *Axonopus compressus*; (c, d) *Parthenium hysterophorus* stained with Chelpark permanent black; (b) *Axonopus compressus*; (e, f) *Parthenium hysterophorus* stained with Chelpark washable royal blue. Arbuscules (Black arrow), Internal hyphae (White arrow) and Vesicles (Double arrow head). Bar = 50 µm

The optimum timing for root clearing are presented in Table 1. The selected inks stained the different fungal structures [1]. Chelpark permanent black stained all fungal structures (arbuscules, vesicles and hyphae) brown to dark brown. Chelpark washable royal blue stained all fungal structures blue. After destaining with lactoglycerol for 15 to 20 minutes there was increased visibility of arbuscules, hyphae and vesicles. Roots not cleared

thoroughly in 10% KOH were difficult to destain due to excessive staining of root tissue, so it is recommended the roots be cleared thoroughly before staining with inks.

Table 1- Optimal timing for root clearing.

| Plant species | Optimal timing for root clearing (min) |
|---|---|
| *Parthenium hysterophorus* | 60-90 |
| *Axonopus compressus* | 45 |

Chelpark permanent black, Chelpark washable royal blue gave good staining results in the selected plant species belonging to Asteraceae and Poaceae, and thus may also be applied to other species within the family and possibly beyond. In this study, due to time and financial constrains only two species of plants representing one family from dicotyledon and one from monocotyledon has been included, and only two inks were tested. It is recommended that the inks be tested on different plants belonging to different families and more locally available inks be included. The present work is confined to plants collected from its natural habitat, so it is recommended that the inks be tested on plants inoculated artificially with different species of arbuscular mycorrhiza.

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