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The Presence of Extrafloral Nectaries (EFNs) in *Pentaclethra macrophylla* Bentham

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Abstract

Extrafloral nectaries (EFNs) mediating ecologically important ant–plant protection mutualisms are especially common and unusually diverse in the family fabaceae. This research work present the first case of the presence of EFNs in the nodes of Pentaclethra macrophylla Bentham detailing and illustrating their locations on the plant, morphology and anatomy. The glands occurred at the node of the plant. Morphological, anatomical and preliminary phytochemical studies were carried out to confirm their presence, structure and function. Interest in EFNs in Pentaclethra macrophylla started after noticing the frequent visits by nectar-seeking ants to the node of the plant. Medi-test strips were used to confirm the presence of sugar. Photomicrographs were made from stained and unstained sections in 50% glycerine. Colour change observed from the test strips indicated the presence of sugar (whose concentration was greater than 500mg/dl). It is recommended that the leaves of six to seven weeks old Pentaclethra macrophylla that has stop producing the exudates should be analyzed for certain metabolites content like tannin and compared to the tannin content on the leaves of a younger plant (two to three weeks old) to see if it is been used by the older plant as a chemical defense against further herbivore attack.

KEY WORDS: African oil bean, extrafloral nectaries, Phytochemical, Anatomical, Insects.

INTRODUCTION

Pentaclethra macrophylla commonly known as the African oil bean is a native of tropical Africa and belongs to the subfamily Mimosoideae and family Fabaceae (Edu and Akwaji, 2017). Is a large size tree with long bipinnate compound leaves that is endemic to West and Central Africa (Olotu, Enujiugha, Obadina, and Owolabi, 2014). It is popular in Nigeria where it is known by several names such as Apara in Yoruba for the seed as well as the fermented product and the most prominent being Ugba (Igbo), as it is a popular condiment and meat analogue among consuming populations (Obi, 2021).The tree thrives in the Eastern and Southern parts of Nigeria. It grows to a height of about 21 m up to 6 m in girth and it is well branched, forming crown-like canopy. It flowers between March and April after which the pods (brown and woody when matured) open by explosive mechanism, dispersing the seeds and curls up. The seeds are dorso-ventrally flattered, hard, brown in colour and about 6 cm long and 3 cm wide (Obi, 2021).

Extrafloral nectaries (EFNs) mediate the most widespread and ecologically important indirect plant mutualistic defence mechanism against herbivores (Heil, 2015). EFNs secrete a carbohydrate-rich nectar reward to attract especially ants, but also other aggressive arthropods, and exploit and rely on them as 'bodyguards' (Kessler and Heil, 2011). These ecologically important ant–plant mutualisms involving EFNs have evolved many times independently in over 100 angiosperm families and some ferns (Weber and Keeler, 2013) and are hypothesised to have spurred plant diversification (Weber and Agrawal, 2014). EFNs are particularly common in the legume family, Leguminosae (Fabaceae), the third largest angiosperm family, which is well known for its rich diversity of interactions with ants. Since then, there has been much renewed interest in exploring aspects of EFN diversity, ecology and evolution in and beyond legumes (Marazzi *et al.*, 2013a) and, equally importantly, the taxonomy of the family has undergone substantial realignments in terms of generic delimitation, and the tribal and subfamily classification, largely as a result of insights from molecular phylogenies (Legume Phylogeny Working Group, 2013 ; 2017).

The vast repertoire of plant defence mechanisms, extrafloral nectaries (EFNs) attract aggressive arthropods that protect developing leaves, shoots and flowers from herbivores (Marazzi, Bronstein, and Koptur, 2013b). Plants in over one hundred families bear EFNs; EFN-bearing species occur in a wide range of habitats and climates and latitudes worldwide, from tropical forests to deserts. EFNs display a remarkable diversity in morphology, density and location on plants, and have diverse ecological functions (Marazzi *et al.* 2013b). They commonly attract ants, as the nectar secreted by EFNs is a valuable, carbohydrate-rich food resource. Other arthropods – some, but not all of which protect the plant from herbivores – also feed on extrafloral nectar. By fostering ecologically important protection mutualisms, EFNs potentially boost the success of certain plant species, thus shaping plant community composition. They are equally important in structuring communities of arthropods, including herbivores, predators and parasitoids (Lach, Tillberg and Suarez, 2010). EFNs-bearing plants occur in tropical and subtropical regions as well as in many temperate regions, including a wide range of habitats, from wet tropical rainforests to deserts (Chomicki and Renner 2015). EFNs may vary in their abundance and distribution on a plant over the course of its development. This temporal dimension is one of the least documented aspects of EFN diversity; very few studies have followed individual plants over their lifetimes, or have examined seedlings at all (Kwok and Laird, 2012 ; Marazzi *et al.* 2013a).

EFNs occurs in a wide variety of plant taxa, particularly among the fabaceae family. In Fabaceae, EFNs has been reported in 105 genera out of the total 730 Genera with 1,094 species out of the total 19,400 species in the Fabaceae family (Marazzi, Gonzalez, Delgado-Salinas, Luckow, Ringelberg and Hughes, 2019). Interest in the study of EFNs in *Pentaclethra macrophylla* Bentham started after noticing the frequent visits by nectar-seeking insects especially ants to the node of the plant. The aim of this research is to study the presence, structure and functions of extra floral nectaries (EFNs) in *Pentaclethra macrophylla.*

MATERIALS AND METHODS

Collection and identification of plant materials

Recalcitrant *Pentaclethra macrophylla* seeds were collected in Choba area around the University of Port Harcourt campus. The Imbibition or Seed Priming method of Leadem, (1997) was used to break the dormancy of the recalcitrant seed of *Pentaclethra macrophylla*, where the seeds were soaked in water for 40 hours before planting.

Materials Used

Potted bags, Soil, light microscope, photomicrographs, meter rule, glass slides with cover slips, digital camera, razor blade, glycerine, capillary tube, medi-test strip, safranin stain and pedri dish.

Methodology

Macro-morphology/ Germination

The leaves, pods and seeds were observed. The recalcitrant seeds were soaked in water for 40 hours according to the method of Leadem, (1997) before been planted and the germination time was monitored for a period of six weeks. The time the node started the nectar was also monitored.

Preliminary phytochemical test for the presence of extra floral nectaries (EFNs)

The presence of nectary gland was confirmed first by using Medi-test strips following the method of Baker and Baker, (1973). This was done by using a capillary tube to take an aliquots of the nectar secreted by the node of the plant before been placed on the test strip and monitored for 60 seconds for colour change which was recorded and the concentration was noted also.

Anatomical studies

About 2-6mm of the nodes of the Stem of a young (4 weeks after planting) *Pentaclethra macrophylla* plant still producing the nectar were obtained and immediately fixed in FAA (Formalin, Alcohol and Acetic Acid) in the ratio of 1:3:1 for 24 hours according to Johasen (1940). Samples were later transferred into (30, 50 and 70%) alcohol for three hours respectively and then into absolute alcohol for 24 hours. These dehydrated samples were infiltrated with wax by passing via diverse concentrations of alcohol and chloroform (3:1, 1: 1, 1:3), as chloroform slowly substituted the alcohol. The schedule of paraffin method described by Johansen (1940) was followed. Thin sections were made using sharp razor blade, Nodal sections were stained with 1% safranin. Photomicrographs were taken using XSZ-N107 Microscope with (MA88-900) camera from stained and unstained sections in 50% glycerine.

Results

Macro-morphology/ Germination

The habit is a tree which has an open crown and a greyish bark (Plate 1), the leaf is sessile bipinnate compound leaf (Plate 2), flower buds are reddish in colour, the inflorescence is a spike. The fruit is a pod which is greyish when mature, with compartments for the glossy brown seeds inside (Plate 3). 50% of the *Pentaclethra macrophylla* seeds began to sprout after 7 days of planting, while the nodes started producing the exudates from the nodes during the second week. The EFNs present in the node of the African oil bean started producing the nectar after the second week of planting (Plate 4), and nectar seeking ants were observed immediately feeding on the exudates (Plate 5). During the fifth week after planting, white mucor-like organism suspected to be a fungus was seen growing on the nectar. But during the sixth week the exudates dries off completely while the organism suspected to be a fungus was still seen growing on the node.

The germination type is hypogeal with a tap root system surrounded by adventitious roots, the plant also can regenerate from previously cut stem of an old plant that had stop producing the exudates. And in the process of regeneration will also produce EFNs (Plate 6)

Preliminary phytochemical test for the presence of extrafloral nectaries (EFNs)

Results from the preliminary phytochemical analysis carried out using the medi-test strip shows a dark green colouration indicating the presence of a very high amount of sugar (reducing sugar), whose concentration was observed to be greater than 500mg/dl. The results of the test strip showing both the control (unused strip) and the used one with colour change (from yellow to dark green) were observed with photographs in (Plate 7).

Anatomical studies: The results shows the presence of extrafloral nectaries (EFNs) as a protuberance at one end of the node of *Pentaclethra macrophylla.* Photographs of both the stained and unstained anatomical structure are contained in plate 8 and 9 respectively. The anatomical structure of the EFNs studied possess epidermal tissue, a secretory zone composed of parenchyma cells with dense cytoplasm and a polygonal cell zone that is lignified with pit fields and vascular bundles, which are composed of phloem and xylem. Calcium oxalate crystals in the form of druses were observed in the parenchyma and in the lignified cells which appeared as dark spots.

Discussion

Interest in extral floral nectaries can arise by noticing the frequent visits of nectar seeking insects, especially ants, wasps and dipterans to the leaves, bracts and probracts of some plants (Okoli and Onofeghara, 1984). Extrafloral nectaries (EFNs) are particularly common in Fabaceae, and their morphological diversity has proved taxonomically useful (Marazzi *et al.*, 2013b). It has been observed that *Pentaclethra macrophylla* possesses extrafloral nectaries at the young stem nodes at about two weeks after planting and this phenomenon accounts for the presence of nectar-seeking insects like sugar ants feeding on the nectar. Baker and Baker (1978) reported the presence of EFNs in the shoot axis of *Pentaclethra macroloba* (Wild.) a related species of *Pentaclethra macrophylla* native to South America. However, the EFNs of *Pentaclethra macrophylla* does not produce the nectar after the sixth week of planting when it is strong enough, indicating that the nectar secretion by the EFNs of Oil bean is for protection against attack by herbivorous animals by using ants that feeds on it as pugnacious bodyguards, thereby supporting the views of the protectionist who have supported the idea that ants visiting the nectaries protects the plants from herbivorous animals. Although matured plants can be made to produce new nectars when it produces new leave during regeneration from the stem that has been cut earlier.

Conclusion

The observations made about the presence of EFNs in *Pentaclethra macrophylla* should serve as a basis for further scientific investigation. EFNs are widespread and evolutionarily labile traits that have repeatedly evolved a remarkable number of times in vascular plants. As suggested by Heil and Mckey (2003), EFNs are plant defenses worn on the outside, and thus analogous to chemical defenses. In view of the fact that extral floral nectaries are known to secrete, in addition to sugars, amino acids and other chemical. Plants with EFNs in both the temperate and tropics are protected from herbivore damage by the nectar-seeking insects visiting the nectaries which has been backed by enormous body of correlative data.

Recommendation

It is clear that EFNs in legumes are strongly concentrated in tropical lineages, with there being few examples of temperate legumes with EFNs (Chomicki and Renner 2015), however, within the tropics, it is unclear whether EFN-bearing legumes are equally abundant across savannas, rain forests and seasonally dry tropical forests, or even deserts. So it is recommended that a research can be conducted on this particular plant species or together with other plant species in the fabaceae family to ascertain the effect of rainfall or water on the production of nectaries. An unknown fungus was also reported to be growing on the nectar secreted by the EFNs of *Pentaclethra macrophylla.* Isolation and identification of such fungus might lead to a discovering of a novel organism.

References

Baker, H. G. and Baker, I. (1973). Amino acids in nectar and their evolutionary significance. *Nature*, 241:543-545.

Chomicki, G. and Renner, S, (2015). Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. *New Phytologist*, 207, 411–424.

Edu, E.A. and Akwaji, P. I. (2017). *Pentaclethra macrophylla* Benth. (African oil bean tree) and *Parkia biglobosa* Jacq (African locust bean tree): The declining giants of the rainforest of Nigeria (The Northern Cross River situation). *World Scientific News*, 64: 127-138.

Heil, M. (2015). Extrafloral nectar at the plant–insect interface, a spotlight on chemical ecology, phenotypic plasticity, and food webs. *Annual Review of Entomology*, 60, 213–232.

Johansen, D. A. (1940). Plant Microtechnique. McGraw-Hill, New York, 523 pp.

Kessler, A.and Heil, M. (2011). The multiple faces of indirect defences and their agents of natural selection. *Functional Ecology*, 25, 348–357.

Kwok, K. E and Laird, R. A. (2012) Plant age and the inducibility of extrafloral nectaries in *Vicia faba*. *Plant Ecology*, 213:1823–1832.

Lach, L., Tillberg, C. V. and Suarez, A. V. (2010). Contrasting effects of an invasive ant on a native and an invasive plant. *Biological Invasions*, 12:3123–3133.

Leadem, C. L. (1997). Dormancy-Unlocking Seed Secrets. National Proceedings, Forest and Conservation Nursery Associations. Gen. Tech. Rep. PNWG TR-419. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research.

Legume Phylogeny Working Group, (2013). Legume phylogeny and classification in the 21st century: progress, prospects and lessons for other species-rich clades. *Taxon*, 62:217–248.

Legume Phylogeny Working Group, (2017). A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. *Taxon*, 66:44 – 77.

Marazzi, B., Conti, E., Sanderson, M. J., McMahon, M. M. and Bronstein, J. L. (2013a) Diversity and evolution of a trait mediating ant–plant interactions: insights from extrafloral nectaries in Senna (Leguminosae) *Annals of Botany*, 111:1263–1275.

Marazzi, B., Bronstein, J. L. and Koptur, S. (2013b).The diversity, ecology and evolution of extra floral nectaries: current perspectives and future challenges. *Annals of Botany*, 111(6):1243-1250.

Marazzi, B., Gonzalez, A., Delgado-Salinas, A., Luckow, M. A., Ringelberg, J. and Hughes, C. (2019). Extrafloral nectaries in Leguminosae: phylogenetic distribution, morphological diversity and evolution. *Australian Systematic Botany*, 32: 409-458.

Obi, V. I. (2021).*Pentaclethra macrophylla* Benth.: A Tree Crop of Potential Economic and Social Values in Biafra Land Research. *Researchgate*

Okoli, B. E. and Onofeghara, F. A. (1984). Distribution and morphology of extrafloral nectaries in some curcurbitaceae. *Botanical Journal of Linnean Society*, 86(2):153-164.

Olotu, I., Enujiugha, V. N., Obadina, A. and Owolabi, K. (2014). "Fatty acid profile of gamma-irradiated and cooked African oil bean seed (*Pentaclethra macrophylla* Benth)". *Food Science and Nutrition*, 2 (6): 786–791.

Weber. M. G. and Keeler, K. H. (2013). The phylogenetic distribution of extrafloral nectaries in plants. *Annals of Botany*, 111:1251–1261.

Weber, M. G. and Agrawal, A. A. (2014). Defense mutualisms enhance plant diversification. *Proceedings of the National Academy of Sciences of the United States of America*, 111:16442–16447.

Plate 1: *Pentaclethra macrophylla* tree showing open crown

Plate 2: Leaf of *Pentaclethra macrophylla* showing the characteristic bipinnate compound leaf

Plate 3:*Pentaclethra macrophylla* greyish matured fruit, with compartments for the glossy brown seeds inside.

Plate 4: Extrafloral nectaries (EFNs). Arrows show droplets at the node of *Pentaclethra macrophylla*

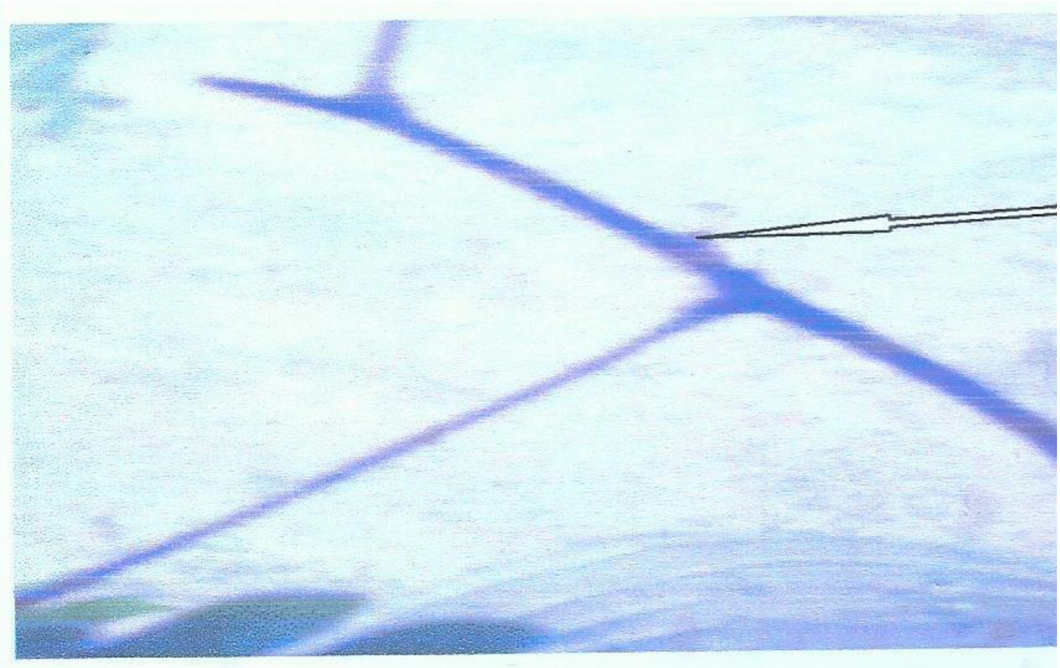


Plate 5: Arrow shows an ant feeding on EFNs of *Pentaclethra macrophylla*

Plate 6: *Pentaclethra macrophylla* plant regenerating from previously cut stem

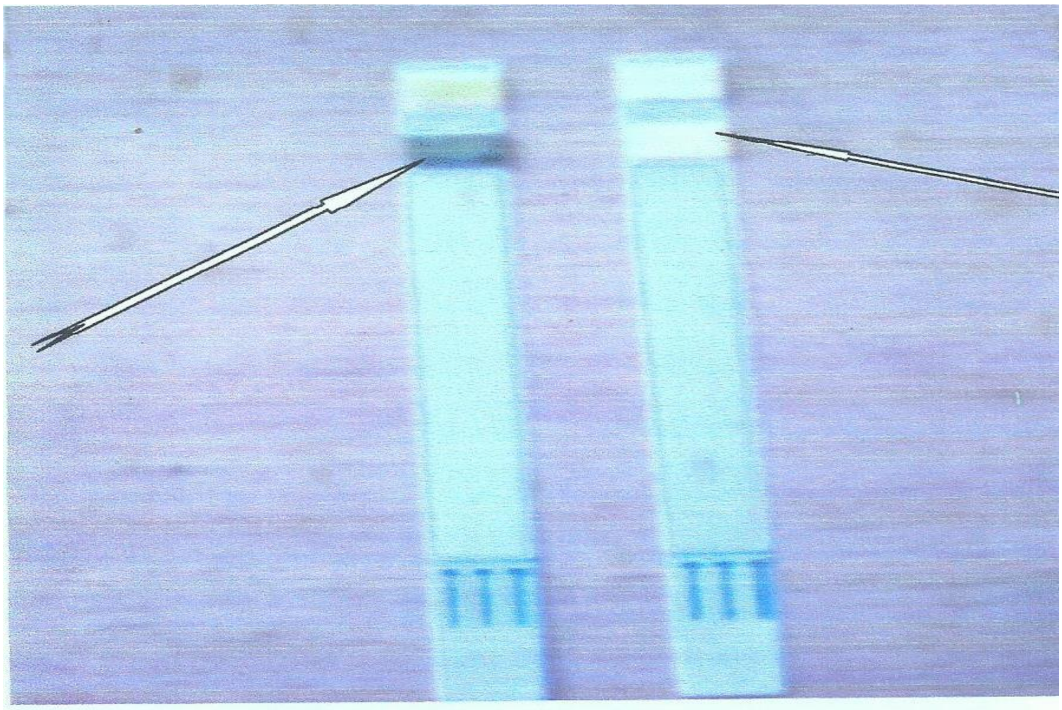


Plate 7: Medi-test strip: Arrows show colour change from yellow to dark green due to the presence of sugar

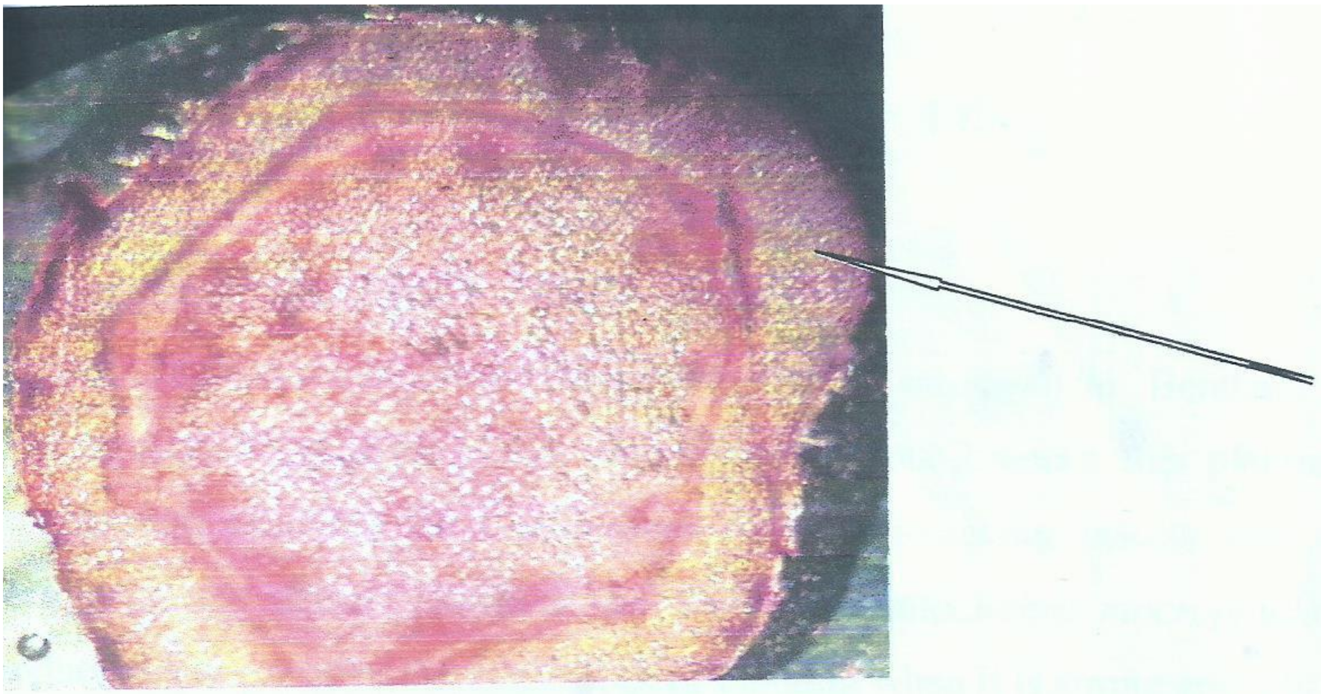
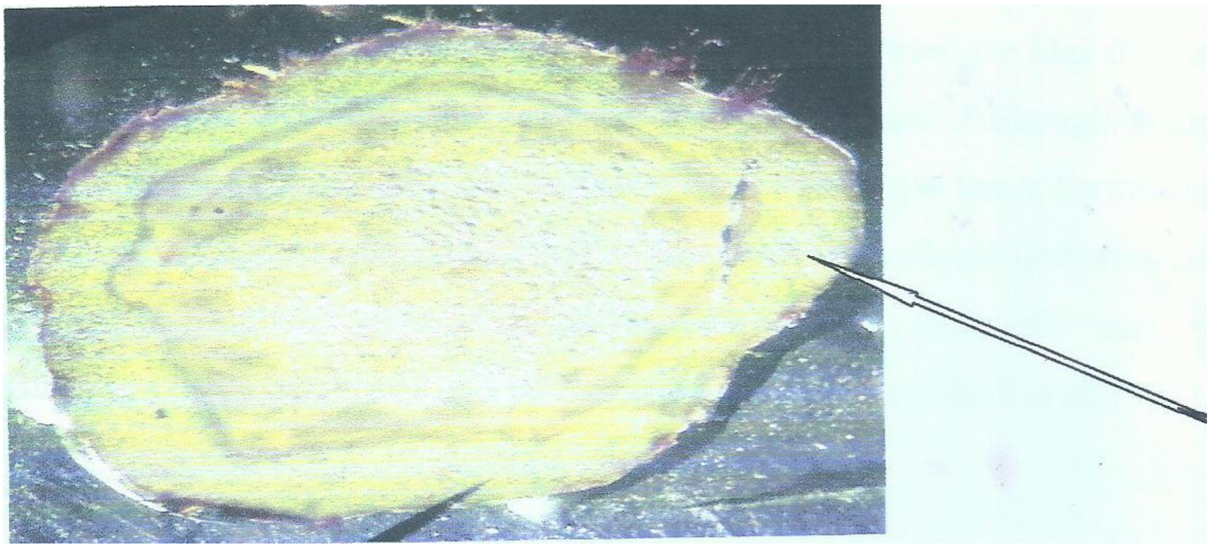


Plate 8: Transverse section of stained stem at the node . Arrow shows the EFNs as protuberance at one end of the node.
Plate 8: Transverse section of unstained stem at the node . Arrow shows the EFNs as protuberance at one end of the node.



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