Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 1078-1081
Received: 10-09-2019
Accepted: 12-10-2019

Dr. Vimal Kumar CS
Former Research Scholar, Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Thiruvananthapuram, Kerala, India

Corresponding Author:
Dr. Vimal Kumar CS
Former Research Scholar, Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Thiruvananthapuram, Kerala, India

Investigation on the *Zaghouania oleae* (EJ Butler) Cummins induced morpho-anatomical modifications in *Olea dioica* Roxb.

Dr. Vimal Kumar CS

Abstract
Olea dioica Roxb, belonging to the family 'Oleaceae' is a tree species locally known as 'Edana'. During the winter season, the plant is seen to be infected with the rust fungus *Zaghouania oleae* (E.J. Butler) Cummins, belonging to the family Pucciniaceae. The fungus infected tender twigs is being cooked and eaten by Chenchu tribal ladies in Andhra Pradesh to beget children. The Preliminary phytochemical screening of the ethanolic extracts of the infected leaves by the author substantiated the tribal claim. In the present investigation, it was focused on morpho-anatomical changes brought out by the infection of the fungus *Zaghouania oleae* (E.J. Butler) Cummins to the host *Olea dioica* Roxb., Fungus induced tissue deformation was observed and the size and shape of the cells were largely affected. The infection of the fungus caused breaks in the epidermis and penetrates the mesophyll layer. At maturity, it turns out to be a cup-shaped Basidium filled with basidiospores. The morpho-anatomical changes brought out by the infection are may be due to the production of secondary metabolites. Most of these compounds might have potential medicinal properties. These findings substantiate the previous phytochemical studies as well as the tribal claim.

Keywords: *Zaghouania oleae* (EJ Butler), morpho-anatomical modifications, *Olea dioica* Roxb.

1. Introduction
All plants in natural ecosystems are thought to be symbiotic with mycorrhizal and/or endophytic fungi [1], and the very existence of the fungi infected plants has been known for over hundred years. Fungi occupy a prominent position in the biological world because of their variety, economic and environmental importance. The association between fungi and plants represents a unique balanced multipartite ecological relationship, which along with other abiotic components of nature forms a complex interplay of the components, which in turn alters the morpho-anatomical features of the host plant. The fungi that live inside the tissues of living plants are an under-explored area of research. Fungus induced morpho anatomical variations are also accomplished with the production of an array of secondary metabolites. These anatomical variations and associated secondary metabolites have proved to be a gold mine to isolate new and novel molecules, useful for the treatment of various ailments. Once the properties of the secondary metabolites are revealed, it would replace conventional molecules and ultimately enhance the health industry.
The fungal flora found on plants exerts their influence on the host plant's morphology, anatomy, phytochemistry, physiology etc. Usually, the host plants overcome the adverse effects of the infection by morpho-anatomical modification and form a complex system with a different class of secondary metabolites. The utility of such metabolites produced by plants infected with different fungi has been proved time and again. The systemic nature of these metabolites results in translocation to different parts of the host [2]. It is also noted that the fungus induced morpho anatomical variation helps the certain host to enhance its biomass production by producing certain growth hormones or inducing the host hormone production pathways [3]. The fungus *Phialocephala fortinii* infecting the plant *Larix decidua* showed that the fungus produces IAA even in vitro and the same induces morpho-anatomical variations and is also responsible for the increase in the biomass production. It is also observed that the fungus induced morpho-anatomical variation equips the plants to tolerate stressful conditions viz., drought, heavy metal tolerance, herbivory, temperature, salinity [4]. This property has been extensively used to develop modern phytoremediation techniques by artificially inoculating the plants with fungal spores [5]. The fungus induced morpho-anatomical changes sometimes also helps to increase the absorptive surface area of the plant due to extra-matrical fungal hyphae exploring rhizosphere beyond the root hair zone, which in turn enhanced water

and mineral uptake. The protection and increased mineral uptake results in greater biomass production. In addition, the phytoalexins produced in the host plant might contain class compounds which are useful and are medicinally significant.

Olea dioica Roxb., belonging to the family 'Oleaceae' is a tree species commonly found distributed in the semi-evergreen and moist deciduous forests in diverse geographical niches of India. The plant is locally known as 'Edana'. Previously, the plant was known for its sporadic use as a medicinal plant. Often, during the winter season, the plant is seen to be infected with the rust fungus *Zaghouania oleae* (E.J. Butler) Cummins, belonging to the family Pucciniaceae [6]. The infection of *Z. oleae* is restricted to leaves and tender shoots causes blister on leaves, hypertrophy, littling, thickening and unusual elongation of the infected shoot [7]. These hypertrophied tender twigs are being cooked and eaten by Chenchu tribal ladies in Andhra Pradesh to beget children (unpublished report, Hosagaudar). Preliminary phytochemical screening of the ethanolic extracts of the infected leaves substantiated the same [8]. In the present investigation, it is focused on morpho anatomical changes brought out by the infection of the fungus *Zaghouania oleae* (E.J. Butler) Cummins to the host *Olea dioica* Roxb.,

2. Materials and Methods

2.1 Collection and identification of fungi

The fungus infected materials were collected from the leaves, soft stems and tender shoots of *Olea dioeca*. Field notes regarding their pathogenicity, nature of colonies, nature of infection, locality, altitude etc. were recorded during collection. For each collection, a separate field number was assigned. The infected plant parts were pressed and dried in between blotting papers. The host and fungus were authenticated by matching it with the herbarium TBGT, Thiruvananthapuram.

2.2 Morphological study

Comparative morphological study of the *Olea dioica* Roxb., infected with *Zaghouania oleae* (E.J. Butler) Cummins both fungus-infected and non-infected plants were carried out. Fungus induced morphological changes were observed.

2.3 Anatomical study

Anatomical studies of both the fungus infected and non-infected leaves of *Olea dioica* were carried out. The leaves were fixed in FAA for 24h. The materials were then dehydrated by passing through tertiary butyl alcohol series. Infiltrations of the materials were done by the gradual addition of paraffin, and the blocks were prepared as per the standard guidelines. The specimens were sectioned using a Rotary microtome. The sections were dewaxed and stained with Saffranine. Photomicrographs of the sections were taken using a microscope with a digital camera attachment.

3. Result

3.1 Morphological changes

The morphological study of the fungus infected and non-infected leaves of *Olea dioica* aimed to evolve the influence of fungal infection on the external appearance of the host plant. The results showed that upon fungus infection, the leaves become folded inward. Infection also caused the formation of blister, hypertrophy, littling, thickening and unusual elongation of the infected shoot.

3.2 Anatamical changes

The present anatomical study of the fungus infected and non-infected leaves of *Olea dioica* aimed to evolve the influence of fungal infection on the anatomical structure of the host plant. The results are furnished as under.

3.2.1 T S of Non-infected leaf

Midrib

TS of the leaf though midrib shows a thick cuticle followed by 2-3 layers of collenchyma cells. Just below thick 1 to 3 layers of chlorenchymatous cells that join the palisade on both sides, the ground tissue is parenchymatous. The vascular bundle to encircled with a sclerenchymatous bundle sheath. The vascular bundle is somewhat triangular lower two sides with slight convex and upper side with concave appearance bundle sheath is followed by phloem and xylem. Pith is large with rounded parenchymatous cells mostly with disintegrated cells.

Lamina

The epidermis of the lamina is constituted by squarish cells and is covered by a thick cuticle. This is followed by 2-3 layers of elongated palisade tissue. The spongy mesophyll consists of 6-8 layers of irregular mesophyll cells with intercellular space. Foliar sclereids with a narrow lumen of different shapes and sizes are present in the lamina especially in the palisade region and near the lower epidermis. Trichomes or glands were not observed on both upper and lower epidermis.

3.2.2 TS of infected leaf

Midrib

TS of the infected leaf through aid showed almost pallicide upper side and rounded lower side. A thick cuticle is present in the outermost layer followed 2-3 layers of collenchymatous cells. Collenchyma is followed by 4-6 layers of chlorenchyma, and ground tissue is parenchymatous. A continuous ring of bundle sheath is present around the vascular bundle, which is followed by phloem and xylem. Vascular tissue cylinder to triangular in outline. Rounded lesions like protuberance seen in the ground tissue as well as in the vascular cylinder probably the fungal infection. Central pith consists of large parenchyma cells. Apothecium/perithecium is seen near the epidermis in the early stages and becomes cup-shaped on maturity.

Lamina

In the lamina, the upper epidermis is with a thick cuticle and is seen ruptured at several places. This is followed by 2-3 layers of palisade mesophyll cells followed by a 4-6 layer of spongy mesophyll cells. Fungal hyphae are seen among the mesophyll and ground tissue cells. Tissues become irregular due to the presence of fungal hyphae.

4. Discussion

Plant-fungal association has been known for long and is of particular interest since the association results either beneficial or harmful. The violation of the morphological and anatomical integrity of the plant tissues under the influence of rust fungi, *Zaghouania oleae* results in the change of various phytochemical parameters, destabilizes the physiological conditions of the host with which it is associated. This in turn makes them more vulnerable to abiotic environmental factors. The present study helped to finding out the influence of

Zaghouania oleae on the anatomical integrity of its host *Olea dioica.*

The study results in the identification of the local and endophytic arrangement of *Zaghouania oleae* in the tissues of the host plant. Under the influence of the fungus, the leaves seem to be folded towards inside and the surface area has been occupied by the rust spores. Fungus induced hypertrophy of the mesophyll cells is observed viz., the palisade and spongy mesophyll cells show significant proliferation. As a result of the tissue deformation under the influence of fungus, the size and shape of the cells are largely affected. These rusts normally have no saprophytic life other than their survival as resting spores. They form semi-internal basidium and sessile basidiospores. The infection of the fungus cause breaks in the epidermis and penetrates into the mesophyll layer. At maturity, it turns out to be a cup-shaped structure. Basidium is external, short thick and rather thick-walled and the basidiospores are sessile and not ejected.

The morpho-anatomical changes in the host plant upon infection of fungus have wide implications. It alters the morpho-anatomical features and also physiology of the plant. This, in turn, evoke the plant to produce such compounds as phytoalexins. Preliminary phytochemical screening of the ethanolic extracts of the infected leaves substantiated the same in which there was an elevation in the phtochemical constituents of the infected plant extract. These compounds in many cases are useful and are medicinally significant. Ultimately the present morpho-anatomical studies substantiate the previous phytochemical studies as well as the tribal claim.

5. Acknowledgement

The author sincerely thanks Dr.V.B Hosagaudar (expired), former Senior Scientist & Head of the Department, Microbiology Division, JNTBGI, and Dr.P.G Latha, Former Director, JNTBGI for valuable advice during the investigation. The author also thanks Dr.Suja S R, Head of the Department, Division of Ethnomedicine and Ethnopharmacology, JNTBGRI and all the staff of the Division for providing necessary facilities and support to successfully carry out the investigation.

Fig 1: *Olea dioica* Roxb., infected with the fungus *Zaghouania oleae* (E.J.Butler) Cummins

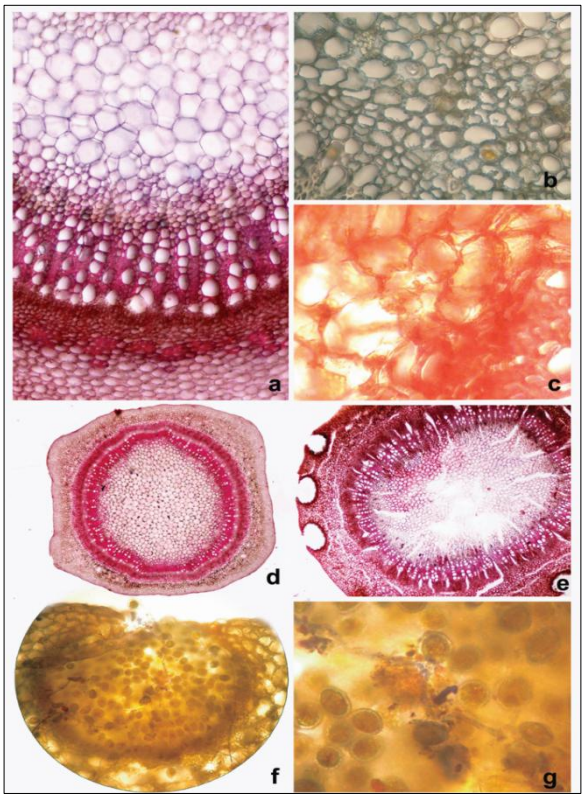

Fig 2: Photomicrograph showing fungus infected and non-infected shoot anatomy a) Non infected shoot b) infected shoot with intercellular myceilium c) deformed cells d) non infected shoot e) infected shoot f) basidium g) basidiospores

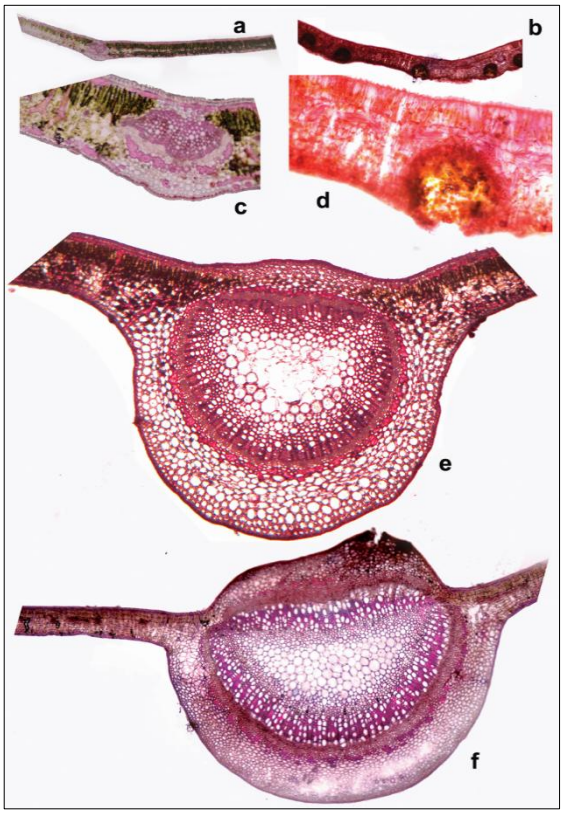

Fig 3: Photomicrograph of fungus infected and non-infected leaves of *Olea dioica*, a,c & e showing normal leaves section, b, d & f showing fungus infected leaves section

6. References

1. Rusty R, Regina R. More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. Journal of Experimental Botany 2008; 59(5):1109-1114.
2. Singh A, Dubey NK. An ethnobotanical study of medicinal plants in Sonebhadra District of Uttar Pradesh, India with reference to their infection by foliar fungi. Journal of Medicinal Plants Research 2012; 6(14):2727-2746.
3. Rommert AK, Oros-Sichler M, Lange T, Aust HJ, Schulz B. Growth promoting effect of endophytic colonization of Larch seedlings (*Larix decidua*) with *Cryptosporiopsis* sp. and *Phialophora* sp., Book of Abstracts, the Seventh International Mycological Congress; University of Oslo, Oslo, 2002, 309.
4. Clement SL, Elberson LR, Youssef NN, Davitt CM, Doss RP. Incidence and diversity of *Neotyphodium* fungal endophytes in tall Fescue from Morocco, Tunisia, and Sardinia. Crop Science 2001; 41:570-576.
5. Hrishikesh U, Sanjib KP, Mrinal KB, Sakhi D. Role of arbuscular mycorrhiza in heavy metal tolerance in plants: prospects for Phytoremediation. Journal of Phytological Research 2010; 2(7):16-27.
6. Cummins, *Zaghouania oleae*. Bulletin of the Torrey Botany Club. 1960; 87:45.
7. Hosagaudar VB. *Zaghouania oleae* (E.J. Butler) Cummins. Journal of Economic and Taxonomic Botany 1988; 12:271.
8. Vimalkumar CS, Hosagaudar VB, Suja SR, Vilash V, Krishnakumar NM, Latha PG. Comparative preliminary phytochemical analysis of ethanolic extracts of leaves of *Olea dioica* Roxb., infected with the rust fungus *Zaghouania oleae* (E.J. Butler) Cummins and non-infected plants. Journal of Pharmacognosy and Phytochemistry 2014; 3(4):69-72.