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Original Research Article

First Report of Fossil Bacterial Lesion on *Citrus aurantium* L. from Late Tertiary Flora of Mahuadanr Valley of Jharkhand, India

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Abstract

Authors demonstrate the presence of gall-like lesion marks in the fossil leaves of *Citrus aurantium* Linn. from Late Tertiary sequence of Mahuadanr Valley of Jharkhand State. Generally the gall lesions are rounded, oval to circular in shape and 1-2 mm in diameter, however, there are some irregular shaped lesions which are more than 3 mm in diameter. They occur mostly near the midrib, in between the margin and in lower as well as in upper portion of leaves. The lesions are open and hollow are surrounded by thick border giving ring effect. The fossil lesions have been compared with the similar infected modern leaves and appear to be similar with the most common gall of *Citrus aurantium*, caused by bacteria *Xanthomonas citri.*

Keywords

Citrus aurantium
Fossil
Gall
Mahuadanr
Tertiary

Introduction

The fossil record of *Citrus* is meager and only known from Late Tetiary of Mahuadanr, Jharhhand, India (Singh and Prasad, 2008). However, there is no information about pathogen associated with fossil leaf. The present article deals with fossil leaf impression having gall caused by *Xanthomonas* in *Citrus* Linn preserved on the lamina of a fossil dicotyledonous leaf. Fossil leaf is collected by Mahuadanr valley, Jharkhand. The Mahuadanr Valley is famous for the occurrence of Late Tertiary exposures containing a variety of well preserved leaf, fruit and seed impressions. The Citrus disease is endemic in India, Japan and other South- East Asian countries, from where it has spread to all other citrus producing continents except Europe (Das, 2003). But they are not known in fossil history. Several workers recorded insect galls in the fossil floras of India like Galls of unknown affinity are described in *Glossopteris* leaves of the Late Palaeozoic age (Srivastava, 1988, 1996; Pant and Srivastava, 1996), however, their records are entirely absent during the Mesozoic. Recent report of unidentified insect galls in the fossil leaf *Sophora benthamii* stem is the only known specimen from the Tertiary flora of India (Srivastava and Srivastava 1998) and a another fossil leaves *Eomangiferophyllum damalgiriensis* (Mehrotra et

al., 1998) from the Upper Palaeocene aged flora of the Tura Formation, north-eastern India (Srivastava et al., 2000). However, the insect galls are well known in contemporaneous floras of Europe and America and have been documented by many previous workers (Straus, 1977; Larew, 1992; Scott et al., 1994). The non availability of such specimens in the Indian floras does not signify their absence; rather, it shows the lack of initiative to examine the fossil flora of the tropical and subtropical region. The species belonging to the families of Fabaceae, Moraceae, Lauraceae, Myrtaceae, Combretaceae, Dipterocarpaceae, Anacardiaceae and Asteraceae bear the insect galls (Docters Van Leeuwen-Reijnvaan and Docters Van Leeuwen, 1926; Mani, 1964; Raman et al., 2005).

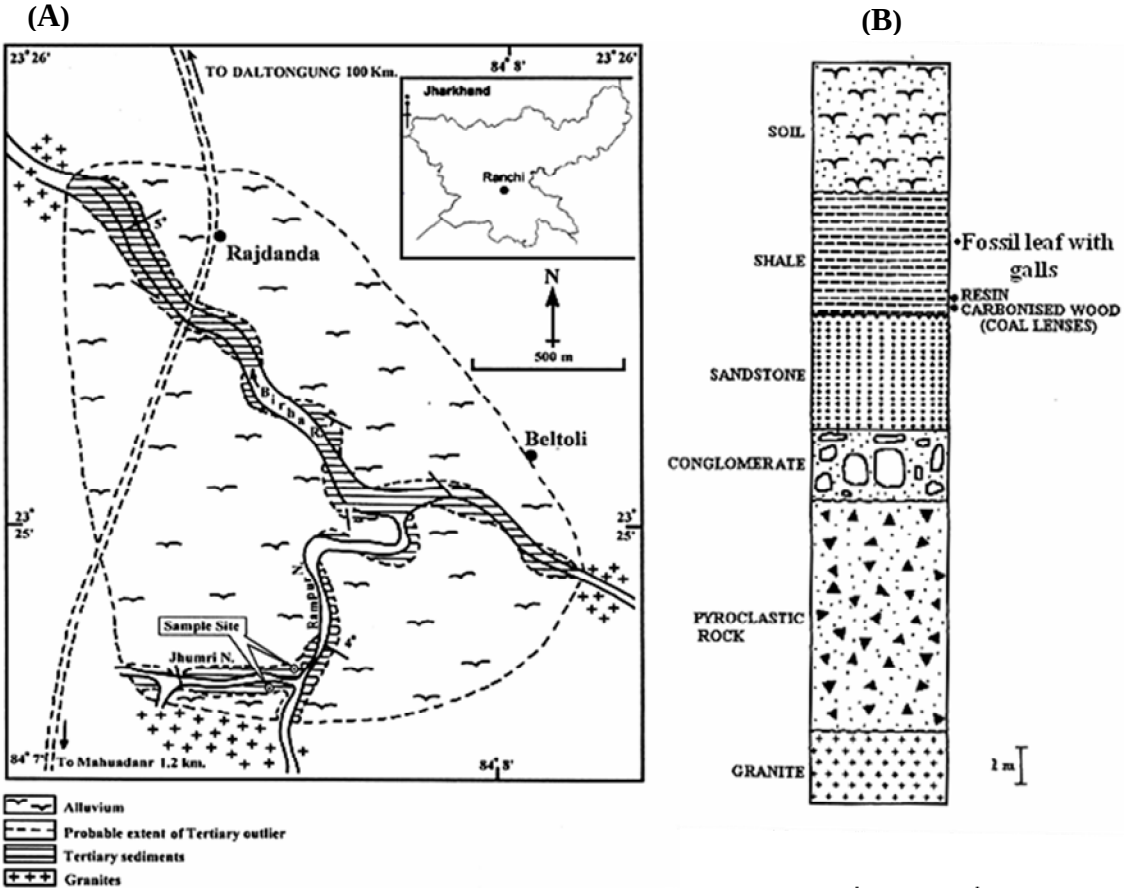
Materials and methods

During our recent survey of Chotanagpur Plateau region of Latehar District, Jharkhand for the collection of megafossil samples, authors collected a variety of leaf impressions from there. They are studied and compared with their modern taxa. In the collection few leaves possesses gall symptoms on their lamina. The gall symptoms were study in detail under low power microscope. The photographs of both fossil leaf and modern leaf having gall symptoms were taken with digital camera. The fossil sample is deposited in Museum of Birbal Sahni Institute of Palaeobotany, Lucknow.

The area of study

The Mahuadanr Valley is famous for the occurrence of Late Tertiary exposures containing a variety of well preserved leaf, fruit and seed impressions. It is situated in the Chotanagpur Plateau region of Latehar District, Jharkhand. The details of the study area are given in Fig. 1. The exposures are located on the bank of Birha river between Rajdanda and Mahuadanr Village (84º 06'N: 23º 23' E) about 116 km south of Daltenganj in Jharkhand. Insect galls are poorly recorded in the fossil floras of the region.

Fig. 1: (A). Geological map of the study area (after Puri and Misra, 1982); (B) Lithocalm of the exposed section at Mahuadanr Valley, Jharkhand indicating the location of fossil leaf with galls.



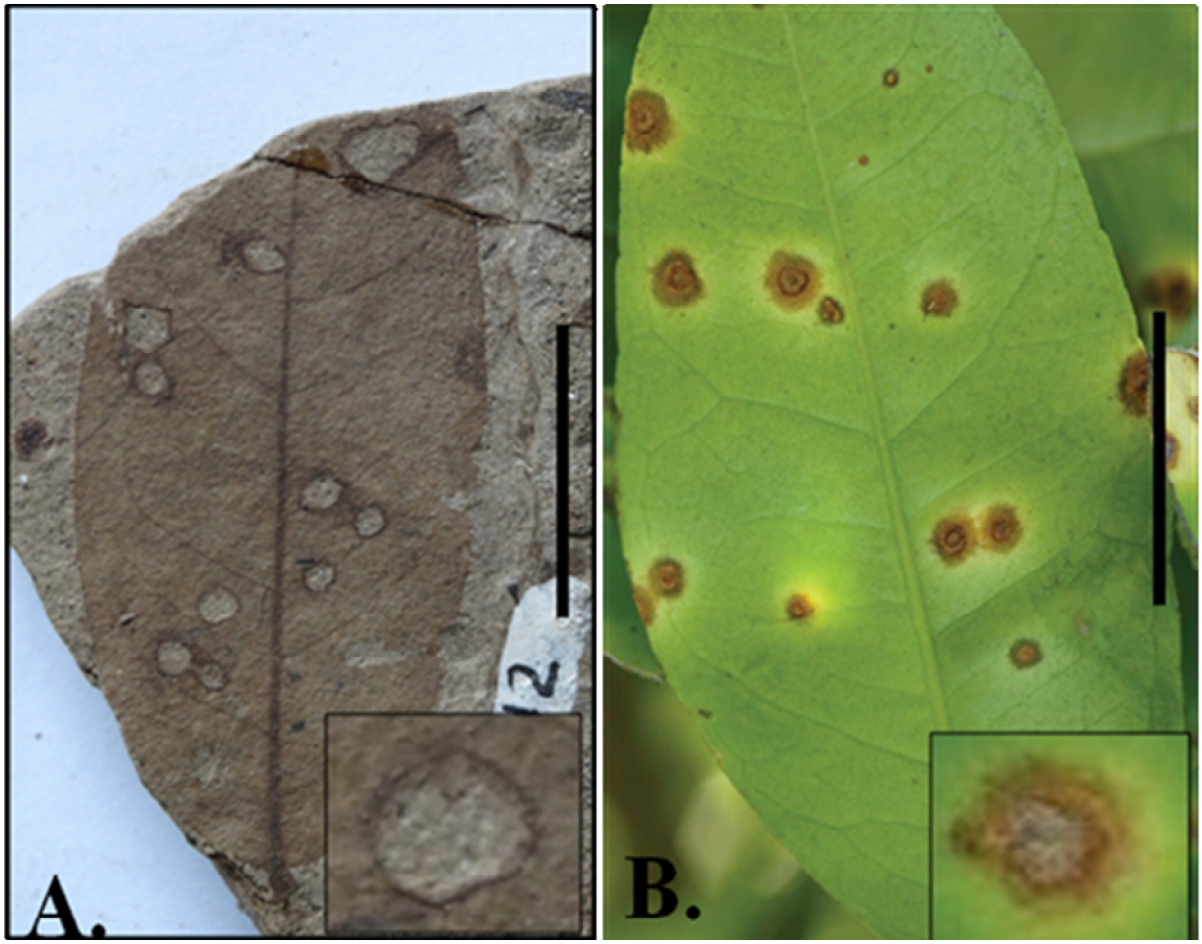
Observations

Affinity between living and fossil *Citrus* gall

Symptoms appear on leaves, twigs, thorns, older branches and fruits. The first visible symptom appear on ventral surface as small, round, slightly raised, water-soaked, translucent spot which, later on , turn white or grayish and finally rupture in centre imparting rough, corky and crater- like appearance. The spots increases in size (1.5-7.0 mm) and generally coalesce to form elongated lesions. The margins of the lesions are sharply defined and are often surrounded by a yellowish halo, but the yellow halo is absent and the crater-like depression in the centre is more prominent in lesion on fruits. However, in severe infection of leaves, twigs and branches weaken the tree.

The survey of literature indicated that the pathogenic bacterium named *Xanthomonas citri* (=*Pseudomonas citri*, = *Phytomonas citri*) is causal agent of Citrus canker disease on Citrus hosts. The causative bacterium is rod-shaped, uniflagellate, aerobic, Gram negative, measuring 1.5–2.0–0.5–0.75µm in size. It forms chains and capsule but does not produce spores (endospores).

Fig. 2: Late tertiary galls on the fossil leaf from Mauadhanr Valley, Jharkhand (A) compared with the leaf galls of modern taxa (B). A. Fossil leaf of *Citrus* sp. showing orientation, distribution and features of galls, BSIP museum no. 39358; B. Modern comparable leaf showing similar details of leaf and leaf galls as fossil (Source-www.google.com).



Fossil leaf and identification (Fig. 2 A)

The characteristic features of the host fossil leaf are median sized (6.6 × 3.0 cm), elliptic shape, normal obtuse base winged petiole, entire margin, eucamptodromous to brochidodromous venation, nature of secondary veins close presence of inter secondary veins undoubtedly suggest its affinity with the leaves of the genus *Citrus* Linn. of the family Rutaceae. In order to find out nearest specific affinity

the extent leaves of all the available species of the genus *Citrus* Linn. were examined and concluded that the leaves of *Citrus aurantium* shows closest affinity with the fossil leaves (Fig. 2B).

Fossil galls: The fossil leaves show symptoms as gall like appearance on lower surface of the Citrus sp. The visible symptoms distributed irregularly either along the midrib or near the margin as well as on lamina of the entire leaf, they are small to large measuring 1.5-7.0 mm in diameter., circular, semicircular some time oval in shape, halo. The severe infection shows the gall like protuberance reach on upper surface (dorsal surface).

Type: Fossil gall of *Citrus aurantium* (T: BSIP 39358), Coll, Sanjai Kumar Singh**,** Mahuadanr valley, Jharkhand.

Geographical distribution

Bacterial canker probably originated in South-East Asia. Subsequently, the pathogen was disseminated throughout Asia, and then to Africa, Oceania and South America (Rossetti, 1977; Commonwealth of Australia, 1984; Stall, 1988). In recent years, the disease has occurred in Islands in the Indian Ocean, in the Middle East and in North America (Das, 2003).

Hosts of *Xanthomonas axonopodis* pv.

Citrus is the one of the important plant of economic importance. Natural infections are known to occur on leaves of *Citrus* spp., as well as on *Poncirus trifoliata*, *Fortunella* spp. (*F. japonica*, *F. margarita*), *Severinia buxifolia* and *Swinglea glutinosa*. In general, grape fruits (*C. paradisi*), limes (*C. aurantiifolia*) and *Poncirus trifoliata* are highly susceptible. Sour oranges (*C. aurantium*), lemons (*C. limon*) and oranges (*C. sinensis*) are moderately susceptible.

Discussion

The structure and nature of fossilized *Citrus* galls though is distinct but as such it is difficult to compare them with the known galls of living counterpart. The lesion is only visible and has no details of their morphological characters. There is no report of galls on the leaf of *Citrus* sp. However, Mani (2000) has reported the presence of galls on shoot axis of *Citrus medica acida* Linn. infected with a fungus *Sphaeropsis tumefaciens* Hedg. from Western Uttar Pradesh.

It is most common disease of citrus plant in their growing areas, regarding as a disease of international importance, was first reported in Japan. The pathogen responsible for citrus canker is *Xanthomonas axonopodis* pv. *citri* (Hartung et al., 1996). Plant galls are cells, tissues or organs of abnormal growth formed due to an increase in cell volume (hypertrophy) and/or cell number (hyperplasia) in response to feeding or other stimuli by foreign organisms (Rohfritsch and Shorthouse, 1982; Dreger- Jauffret and Shorthouse, 1992; Raman et al., 2005). A new definition of galls was coined by Raman (2007) excluding plant growth induced by bacteria and fungi, which form amorphous growths that are called tumors.

In present study, lesions were open and hollow but characteristically each lesion is surrounded by thick border giving ring effect and irregular in shape. Canker spots on leaves, become corky and more irregular in shape. Due to cankerous appearances surface margin completely disappears. Generally, *Xanthomonas*, lesion size among citrus types ranged from 2.0 to 3.5 mm in diameter (Gottwald, 1993), but in present findings also the lesion diameter ranges from 1 to 3 mm. Lesions acquired a crater like appearance is more noticeable. Similar characteristics were noticed in studied impression of citrus leaf.

Mani (2000) has described same features of leaf gall of *Memecylon edule* Roxb. due to an unknown Eecidomyiidae from Travancore and West Bengal. In leaf, mature galls with irregular patches of corky layer on the surface with irregular and more or less deep fissures, but in which generally a profuse growth of sooty- black fungus are noticed. In present communication there is no report of fungus or insect related structure.

Therefore, on the basis of morphological symptoms, it can be concluded that *Xanthomonas citri* Linn. is the pathogen of studied citrus leaf impression, is a host specific bacteria (Gottwald, 1993). The disease is characterized by erumpent lesions on leaves, stems and fruits of *Citrus* species (Cason et al., 1978) followed by hypertrophy and hyperplasia (Gramham and Gottwald, 1991). All these morphological characters have been observed during the study of leaf impression.

Conclusion

The plants that sustain us on this earth evolved much earlier than the first man. However, before the origin of plants, microbes had already developed in the organic evolution. Thus, the association of microbes with plants is much older than association of man with plants. This has significance in the light of adjustment or disadjustment between plants and microbes during their coevolution. In the beginning of this relationship, the microbes started living on dead organic substrates left by plants since they had no faculty to sythenthesize their own food. With further evolution, these microorganisms gave rise to parasitic forms which are even today attacking our crops and disturbing our crop production programmes.

Various diseased twigs and leaves provide inoculum for disease development. The pathogen enters the host via different types of wounds, natural opening etc. On penetration into the host it multiplies in the intercellular spaces, dissolves the middle lamella and establishes itself in cortex. Cankerous outgrowths now develop within which bacteria multiply and released with exudations. The disease is chiefly disseminated by wind, rain and insects.

The present report of Citrus infected gall also provides the additional supportive evidence of climatic condition. It has been found that an atmospheric temperature of 20-30°C, with an optimum of about 30°C and free moisture of about 20 minutes or more on the surface of rapidly growing tissue, are essential for the infection of leaves. Infection is related to the density and maturity of the stomata, being heaviest during their development (Rao and Hingorani, 1963). It is also concluded that host and pathogen are coevolved during the biological evolution.

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