SANDAL SPIKE DISEASE

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ABSTRACT

A review of the work done so far on the spike disease of sandal (*Santalum album* Linn.) has been made under the heads, symptoms of the disease, causal organism, collateral hosts of the pathogen, rank vegetation and disease incidence, vectors for the disease, culture and serology, physiology of the spiked sandal and attempts at controlling the spike disease.

SANDAL (*Santalum album* Linn.) is one of the most economically important forest species in India and is the source for the world famous "East Indian sandalwood oil". The species has a limited distribution in the world, confined to India and Indonesia. In Indonesia, the species occurs in four islands around Timor. In India, though it is mainly concentrated in the Deccan Plateau, it is also found in small numbers almost in all States except the Himalayas and other cold areas. The largest area of natural sandal is found in the southern parts of Karnataka and the northern parts of Tamil Nadu accounting for nearly 90% of sandal in India.

Sandal is a small evergreen tree growing to a height of 10 to 15 m and a girth of 1 m, though trees of 20 m in height and 2 m in girth are also occasionally found. The tree flourishes well from sea level upto 1800 m altitude in different kinds of soils and climates except in highly alkaline or water logged or very cold places. The tree is a hemi-root parasite with a wide range of host plants[1]. The absolute necessity of host, however, is yet to be established. The tree generally flowers twice a year once in July-August and again in November-December.

SPIKE DISEASE OF SANDAL

Sandal is subject to only a few diseases of which the spike disease is the most destructive and has attracted wide attention. Spike disease of sandal is a "Yellows type of disease" showing 'witches' broom effect.

The disease, first reported[2] in Coorg in 1899, is now found confined to the southern parts of Karnataka and northern parts of Tamil Nadu. Recently this disease was found to have spread to Marayoor in Kerala. In natural forests radial spread of the disease is commonly encountered. Isolated distant spread is rather rare. Trees of all ages and sizes are attacked by the disease and the diseased trees die within a few years after the onset of the spike disease.

1. Symptoms of the Disease

Two types of sandal spike have been reported; the more common form has been designated "Rosette spike" but usually called merely spike disease, while the second is known as "Pendulous spike"[3]. The most characteristic symptoms of spike disease are the severe reduction of the leaf size and the shortening of the internodes so that the leaves become too crowded on the leaf-bearing branches. As the disease advances, the fresh leaves become smaller and smaller and during this change the lateral decrease is much more than the longitudinal one. Simultaneously, the leaves show a tendency to stand out stiffly from the branches like spike, from which property, the disease derives its name. Another characteristic feature of the disease is that, as it progresses, the leaves become yellowish and finally reddish shortly before the death of the tree. The symptoms do not normally appear simultaneously all over the affected tree; they start with certain parts and gradually spread over the entire tree. The flowers of the infected tree show phyllody; the diseased parts rarely bear any fruit, while the unaffected parts often flower and fruit well. In the diseased sandal, there is the death of root ends and haustorial connections[2]. Also, in the diseased sandal there is phloem necrosis, multinucleate condition and degeneration of plastids[4] and cytoplasmic inclusions are observed in the cells associated with phloem and xylem tissues[5]. Pollarding, the sandal forces out the spike disease if the disease is remaining masked in the tree.[6]

The characteristic feature of the "Pendulous spike"[3] is that the individual infected shoots show continuous apical growth, growing disproportionately along the length in contrast to thickness and hence they assume a drooping habit. Further, the dormant buds do not develop or grow with the result that the branching and clustering of shoots, characteristic of the rosette type of the disease are absent. Another characteristic of this disease which distinguished it from the rosette spike, is that the root ends and haustoria do not die. The other pathological symptoms are very similar to those of rosette type.

2. Causal Organism

The spike disease was formerly thought to be viral in nature from the disease syndrome and the graft-transmissibility of the disease[7]. Transmissibility of the

disease was further confirmed by bud and patch graft, leaf insertion[8] and through dodder transmission[9]. Sap transmission of the disease was reported by Nayar[10].

Recently through electron microscopic examination, it was shown that the spike disease is caused by mycoplasma-like organisms (MLOs) which are found in the sieve tubes of spike infected sandal leaves and twigs[11-13]. Remission of the disease symptoms by tetracycline treatment confirmed the mycoplasmal etiology of the spike disease.[14-16] Electron microscopy of graft-transmitted diseased leaf also showed presence of MLOs[17]. Electron micrographs of tetracycline treated spiked leaf showed either moribund MLOs or none at all[14]. The morphology of the MLOs in spiked sandal showed marked similarity with the one seen in plants infected with yellow diseases[18,19]. The size of the MLOs ranged from 60–750 nm, the most commonly occurring type being an ellipsoid of about 180–220 nm × 250–300 nm; some elongate bodies (up to 750 nm × 150 nm) were observed. The ribosomes in the sandal spike MLOs were estimated to be about 14–15 nm across and were smaller than those normally found in the cell cytoplasm; they occurred as random components or small aggregates without regular array. Thus the MLOs were pleomorphic with varying shapes and sizes, and no evidence of a cell wall component was visible. The outer envelope consisted of a smooth unit membrane measuring approximately 10–12 nm across[12].

3. *Collateral Hosts of the Pathogen Causing Spike Disease*

Little leaf symptoms resembling sandal spike were reported to have been found in a number of plants *e.g. Zizyphus oenoplea, Vinca rosea, Dodonea viscosa, Eucalyptus grandis, Stachytarpheta Jamaicensis, Dendracalamus strictus* etc. growing along with spiked sandal trees[20]. The disease could be transmitted from the diseased *Zizphus oenoplea and Vinca rosea* to healthy sandal and vice versa through dodder[9]. Electron microscopic examination also revealed the presence of MLOs in the leaves of some of these plants[14]. *Lantana camara* has been found to be a symptomless carrier of the disease pathogen[21]. As the spike disease is transmitted through root contact[22] and insects[23] the presence of these alternate sources of infection makes the disease control operation highly complex.

4. *Rank Vegetation and Disease Incidence*

Rank vegetation associated with sandal was found to have a significant role in the spread of the spike disease in nature. It has been observed[24,25] that the incidence of the disease is high in scrub jungles where rank vegetation is dense and is low in pole forest where undergrowth is scanty. Lantana was earlier considered as the pre-disposing factor to spike disease and experimental removal of undergrowth like *Lantana* and diseased sandal trees, allowed the healthy sandal trees to remain disease free for a considerable time[26].

5. *Vectors for the Spike Disease*

Many of the yellows type of plant diseases are persistently transmitted by insect vectors[27]. In the case of sandal spike disease, two species of leaf hoppers namely *Moonia albimaculata* and *Coelidia indica* (*Jassis indicus*)* were incriminated as possible vectors transmitting the disease in nature[23,28]. In the insect transmission studies recently carried out, *Nephotettix virescence* has also produced spike like symptoms which could also be successfully transmitted to healthy sandal plants by leaf grafting, thus confirming disease transmission by *N.virescence*[29]. Thus more than one species of leaf hoppers seem to be involved in spreading the disease in nature.

A study of the types of bacteria, fungi, actinomycetes and nematodes present in the soils from healthy and spiked sandal regions and their ability in serving as vectors for the disease, revealed that none of these organisms served as vectors for the spike disease[30].

6. *Cultural and Serological Studies*

The mycoplasma-like organisms causing spike disease in sandal could be cultured in a semi-synthetic medium and inoculation of the cultured pathogen into 2-year old healthy sandal plants and *Stachytarpheta indica* plants (pot cultures) could produce spike disease symptoms in them[31]. An antiserum was obtained for the spike pathogen which offers a specific means for the detection of the disease[32]. A study of the amino acid metabolism in pure cultures of the MLOs causing spike disease, showed that the mycoplasma probably utilises glycine, isoleucine, methionine, histidine, aspartic acid, glutamic acid and cystine for its growth[33].

7. *Physiology of the Spiked Sandal*

Considerable work has been done on the physiological and biochemical aspects of the spiked sandal. The salient findings made so far are presented below.

Mineral metabolism

In the spiked sandal, since the haustoria die, thus

*Recent examination of the material formerly identified as *Jassus indicus* shows that they are not *J. indicus* but belong to a new genus and species, and *J. indicus* does not occur in South India.

cutting off the channel of nutrition from the host plants, the mineral composition of the diseased sandal can only be expected to considerably change. From his experiments using potted sandal plants growing alone and in association with host, Sreenivasa Rao[34] expressed that sandal depends on its hosts, for its requirement of N, P, K and derives its other mineral constituents including Ca, Fe and Al from soil. On the other hand, Varadaraja Iyengar[35] inferred from his experiments that sandal drew Ca and K directly from the soil and N and P from the host plant. Ramaiah *et al.*[36], in a histochemical study of the sandal root haustoria, found the nutrients K, Ca, Mg, Fe, Cu, and Zn in the vascular strands of the haustorium thus suggesting that these minerals are taken by the sandal from the host plant. The possibility of absorption of these nutrients directly from the soil also is very much in evidence as sandal roots do possess cation exchange capacity at a level comparable to the levels occurring in any of its hosts and this capacity is in no way affected in the diseased state[37,38]. The observation that there is a considerable decrease in the ash, K_2O, P_2O_5 and CaO contents in the leaves and stem of the spiked sandal[39,40], indicates that a good part of the mineral nutrients is derived from the host through haustoria which die in the spiked sandal.

Chlorosis of spiked leaves

In the spiked sandal, the level of iron in the leaves is reduced while it increased in the stem and root regions, suggesting a disturbance in the translocation of iron from the roots to the shoots in the diseased sandal[41]. The reduced level of iron has a bearing on the chlorosis of the spiked sandal leaves. Further, in the diseased leaves, initiation of loss of chloroplast structure, even at mature stage and increase in chlorophyllase activity (hyrolytic) at senescent stage were contributory for the chlorosis at these stages[42]. At the young and fairly grown up stages of the leaves (healthy and spiked), wherein chlorophyllase activity increased concomitant with chlorophyll accumulation, a comparatively lower level of chlorophyllase activity (synthetic) in the spiked plant seemed to explain the low chlorophyll level in the diseased leaves at these stages[42].

Carbohydrate metabolism

One of the prominent characteristics of spiked sandal is an abnormal accumulation of starch in the diseased leaves. Also the level of sugars was found to increase in the diseased plant[40]. An increased level of diastatic activity in the spiked leaves[43–46] could not apparently explain the accumulation of starch in these leaves. A lowering in the phosphoglucomutase activity in the spiked leaves, however, seems to be a cause for the starch accumulation therein[47].

Nitrogen metabolism

Narasimhamurthy and Sreenivasaya[48] found in the spiked leaves an increase in total N, soluble N, basic N and amino N and a decrease in protein N which suggested an increased rate of degradation of proteins in these leaves. Parthasarathi *et al.*[49], found an increase in the nitrate reductase activity and nitrate N in spiked leaves. Since molybdenum is a metal constituent of nitrate reductase and since any deficiency of this element leads to a considerable decrease in the activity of this enzyme, it is apparently clear that no deficiency of this element exists in the diseased plants. It may be mentioned that a study of the trace elements, Cu, Zn, Mn, Mo and Co in the soils of the healthy and spiked sandal areas has shown that no deficiency of any of these elements serves as a pre-disposing factor for the onset of the spike disease[50].

Nucleic acids, nucleases and nucleotidases

A marked decrease in the ribonucleic acid (RNA)/deoxyribonucleic acid (DNA) ratio in the young leaves of spiked sandal indicated a serious derangement in the nucleic acid metabolism therein probably due to multiplication of the infective principle at that stage. Relative variations in DNA and deoxyribonuclease activity indicated a possible migration of the spike pathogen in the spiked plant from the mature leaves to regions of fresh vegetative activity[51]. In the young diseased leaves an increase in 5′ nucleotidase activity was noted indicating a possible adverse effect on the level of ribonucleoside triphosphates serving in the energy transfer mechanism of the cell[52]. Also, compared with the mature leaves, a relatively higher level of 3′-nucleotidase activity in the young leaves of both healthy and diseased plants was noticed which could be correlated with the high level of ribonuclease activity noticed in them[52].

Polyphenolase, polyphenols: Respiration, hormonal imbalance:

Polyphenolase was found to play a part in the respirational processes in the spiked leaves. A high level of both polyphenols[53] and polyphenolase activity[54] seemed to be responsible for a low level of indoleacetic acid oxidase (IAA oxidase) activity[55] and a high level of IAA in the young spiked leaves. This appeared to explain the generation of a large number of buds in the spiked sandal. The diminutive growth of the spiked leaves, however, seemed partly to be the result of a low level of Ca and high level of ascorbic acid[56,57] in these leaves.

Organic acids

Varadaraja Iyengar[58] found a specific accumulation

of succinic acid and a decrease in the malic and oxalic acid levels in the spiked sandal leaves. Ramaiah *et al.*[59] noticed considerable accumulation of pyruvic acid while the level of oxaloacetic acid was almost negligible in the spiked leaves; from this it was opined that the smooth functioning of Krebs cycle is possibly affected in the diseased sandal.

Identification of spike disease in doubtful cases: Physiological Index and stain tests:

Varadaraja Iyengar[60] suggested that the Ca/N ratio in the leaves could serve as an index for the diagnosis of spike disease, the ratio being approximately 1·2 for the healthy and 0·3 for the spiked leaves. This author also suggested that the length of leaf/breadth of the leaf ratio (l/b ratio) could serve as an index for the diseased sandal, the ratio being 2·0–2·7 for the healthy and 3·6–4·9 for the spiked. However, this ratio could not be of use in respect of new flush of healthy sandal where the ratio was found to be 4 to 7 thus overlapping the ratio for the spiked sandal. Parthasarathi *et al.*[61] developed three stain tests for the identification of the spike disease in doubtful cases. These tests include the use of Mann's stain, carbolthionin and Giemsa stain.

Free hand sections of the apical stem portion of the twigs from healthy and spiked sandal plants were used for the tests. The colour variations obtained with the tests are shown in table 1.

TABLE 1

Stain tests for sandal spike disease

	Healthy	Spiked
Mann's stain	Pink	Pink throughout, except the phloem region which was coloured violet.
Carbol thionin	Blue	Deep violet
Giemsa stain	Light Bluish green	Bright blue

The colour variations produced can be observed with a hand lens or even with the naked eye. These tests developed for the identification of the spike disease in sandal are found to be applicable even in the case of the little leaf disease caused by mycoplasma-like organisms in *Vinca rosea, Zizyphus oenoplea, Eucalyptus citriodora* and *Dodonea viscosa*[57].

8. Attempts at Controlling the Spike Disease

i. Heat therapy

Nayar *et al.*,[16,62] reported that the MLOs causing spike disease grow best within a temperature range of 30–38°C *in vitro*. Below and above this range the growth of the organism slows down. Control of spike by heat therapy by building controlled fires in trenches around the spiked sandal tree, was not successful because of the sensitivity of sandal trees to high temperature and the practical difficulties attending on such a measure in the natural forest.

ii. Chemotherapy

Like many plant diseases of mycoplasmal origin, spike disease of sandal was also seen to respond to treatment by antibiotics belonging to tetracycline group[15,16,63]. Also, benlate, a systemic fungicide, was reported to bring about temporary remission of the disease symptoms in the spiked sandal[64], Rao *et al.*[63] reported that, using the girdling method of application of the antibiotic, spiked sandal trees showed recovery in symptoms 25–30 days after treatment with terramycin alone or terramycin + benlate, 30–35 days with ledermycin alone or ledermycin + benlate; it took longer time in treatments with achromycin, aureomycin or benlate. The recovery lasted for 120 to 150 days in the mixed treatments whereas when treated with ledermycin or terramycin alone it lasted 50 to 60 days, with benlate 80 to 90 days and with achromycin or aureomycin 39 to 40 days. Nayar *et al.*[16] found remission effect in the spiked sandal plant by treatment with achromycin, aureomycin and ledermycin; ledermycin was most effective in that normal foliage was produced in a lower dose than the other two antibiotics. However, the remission effect of the antibiotic treatment lasted only for a limited period. Nayar *et al.*[65], by bioassay, indicated quantitatively the extent of tetracycline uptake and retention. Tetracycline treatment of the spiked sandal could not provide a lasting remedy for the spike disease.

iii. Removal of sources of infection

Prior to 1927, in the Mysore State, attempts were made at controlling the spike disease by uprooting infected trees and by isolating the affected tracts by clearing all sandal growth in a strip of 100 yards around the affected patch of sandal trees. But no beneficial effect of this measure could be seen on the spread of the disease in the field[66]. Similarly, efforts were made in the Madras State to control spike disease by cutting a wide belt around the spiked sandal tree patches and by killing the spiked sandal trees by arsenicals[67]. But this measure did not prove useful either in arresting the spread of disease or in controlling it.

iv. Disease resistance through host selection

Sandal associated with certain hosts was considered

more susceptible to the spike disease than others[68]. It is however seen that resistance through host selection is not possible.

Finding a spike disease resistant strain of sandal and biological control of the insect vector(s) responsible for the spike disease transmission, are the twin pillars on which a permanent solution for the problem of sandal spike rests. Work on these lines is in progress.

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EFFECTS OF ONION IN INDUCED ATHEROSCLEROSIS IN RABBITS: II. REDUCTION OF LIPID LEVELS IN THE EYE†

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ABSTRACT

The influence of onion extract on the lipid levels of the eye in hypercholesterolemic rabbits has been studied and a comparison has been made with rabbits on cholesterol enriched diet alone and with normal controls. The results show that the total lipids, cholesterol and phospholipids are significantly higher in animals on cholesterol diet than in animals on cholesterol and onion extract diet. The lipid levels in the latter group are comparable to the levels of the control group.

THE ocular damage during hypercholesterolemia has been observed in rabbits and human subjects. In rabbits the lipid deposition in the iris appears earlier and is more conspicuous than the involvement of cornea[1-3]. Similar changes involving the deeper structures of the eye have also been reported[4-7]. Ocular symptoms such as acute juvenilis and lipid keratopathy have been observed during familial hypercholesterolemia with massive snowball like exudation of cholesterol in vitreous[8].

The effects of various regressive agents have generally been reported on the aortic lesions and the associated lipid levels[9-14], but to our knowledge the effects of these regressive agents on the eye lipid levels have not been reported. Therefore, in the present study the effect of onion extract, which reduces significantly the aortic lesions and lipid levels[15], maintaining the normal shape of erythrocytes at the varied levels of plasma constituents[16-19], on the lipid levels in the eyes of the rabbits fed with hypercholesterolemic diet, is determined and compared with the normal controls.

White albino rabbits of same age, weight and sex are fed with normal diet (carrot, cabbage and greens) and divided into three groups (ten animals in each group). Group I served as normal control; Group II rabbits are fed with atherogenic diet (normal diet plus 0·5% cholesterol) and Group III are given atherogenic diet as above and extract of 20 g fresh onion, prepared by the method of Stoll and Seebeck[20]. Plasma cholesterol levels are maintained at 1000–1400 mg% by the dietary adjustment of cholesterol. At the end of six months, the rabbits are sacrificed.

The total lipids are extracted by the method of Folch *et al*[21]. Plasma and eye cholesterol are determined by the method of Abell *et al*[22]. The lipid

†This work was carried out at the Biomedical Engineering Division, Indian Institute of Technology, Madras, India.