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Research Review Article

Status of coconut basal stem rot disease in India – A review

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ABSTRACT

Basal stem rot is one of the important diseases of coconut accounting to severe yield loss in southern India. The disease in Indian subcontinent is reported to be caused by *G. lucidum* (Leys.) Karst., *G. applanatum* (Pers.) Pat. and *G. boninense*. Present status of the disease pertaining to occurrence and distribution in major coconut growing states, symptomology, etiology and epidemiology, disease indexing, pathogen diversity and its management methods are reviewed. The disease if unattended, is becoming a major threat to coconut production in Andhra Pradesh, Karnataka and Tamil Nadu states. Management of the disease is possible with continuous monitoring and implementing biocontrol based integrated management even though there is variation among the pathogenic virulence of *Ganoderma* isolates. As early disease escapes detection, recent developments in early detection, grouping and molecular identification of the pathogen and integrated disease management measures are summarized in this review paper.

Key words: Basal stem rot, Coconut, Etiology, Management, Symptomatology

Coconut (*Cocos nucifera* L.) is an important plantation crop in India. It provides nutritious drink, many edible nutritious products, oil for edible and non edible uses, fibre of commercial value, shell for fuel and industrial uses, beverage, timber and a variety of miscellaneous products for use such as handicrafts etc., and contribution of coconut to the GDP of the country is around ₹ 10 000 crores. India ranks third in area, first in production and productivity in world coconut scenario and being grown in an extent of 19.7 lakh ha area with a production of 20 439 million nuts (CDB 2015). Southern states such as Tamil Nadu, Kerala, Karnataka and Andhra Pradesh are the major producers of coconut contributing for 88.8 % of total area and 91.2 % of total production in the country. Coconut palm is an essential and dominant component of the homesteads and garden lands along the coastal parts of southern India and it plays a vital role in the sociocultural and economic life of large number of small and marginal farmers (Dagar *et al.* 2014). Of the total production in the country, 50% is utilized for household consumption, 15% as tender coconut, 35% is converted to copra along with other produces such as virgin coconut oil, desiccated coconut power, coconut milk/cream, etc. (ICAR-CPCRI).

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Diseases play an important role in palm loss and reduced yields of coconut in India. Even though coconut palm is hardy in nature and adaptable to varied climatic conditions, it is affected by many diseases (Nambiar 1994, Henry Louis 2002). Root (wilt) (*Phytoplasma*), basal stem rot (BSR) (*Ganoderma* spp.), bud rot (*Phytophthora palmivora*), stem bleeding (*Thielaviopsis paradoxa*), leaf blight (*Lasiodiplodia theobromae*) and grey leaf spot (*Pestalotiopsis palmarum*) are the major diseases of coconut in India. Among them, basal stem rot (BSR) disease caused by *Ganoderma applanatum* and *Ganoderma lucidum* is the most destructive disease accounting to severe yield loss in southern parts of India. The disease is also known as *Ganoderma* wilt (Andhra Pradesh) or Tanjavur wilt (Tamil Nadu) or Bole rot or anabe roga (Karnataka) in different parts of India (Naik 2001).

Research efforts by many scientists across the country resulted in identifying etiology of the disease, steps in symptom development, role of soil and weather factors on the disease and how to manage or contain the disease using chemicals and bioagents. A good effort was also made by some scientists on reviewing the disease status and its development from time to time (Bhaskaran *et al*. 1989, Srinivasulu and Rao(2007). The present review paper updates the status of BSR in India along with recent developments in early detection, molecular identification and integrated disease management methods and to identify research priorities and knowledge gaps.

OCCURRENCE AND DISTRIBUTION

In India, *Ganoderma lucidum* on coconut was first recorded in Karnataka state during the year 1913 by Butler (Petch 1916). Similar disease symptoms were reported to

be caused by *Ganoderma applanatum* and *Ganoderma lucidum* in Andhra Pradesh by Papa Rao and Govinda Rao (1966) and Satyanarayana *et al.* (1985). In Tamil Nadu, the disease as Thanjavur wilt was first noticed in Thanjavur district after the 1952 and 1956 cyclones (Vijayan and Natarajan 1972). Occurrence of basal stem rot disease similar to Thanjavur wilt of Tamil Nadu was reported in Kerala on coconut by Wilson *et al.* (1987). Peries (1974) reported *Ganoderma boninense* as causal organism of basal stem rot in Sri Lanka. The disease was reported as a major limiting factor in coastal districts of Tamil Nadu and Andhra Pradesh (Vijayan and Natarajan 1972, Srinivasulu 2001a).

Ganoderma species cause significant damage to coconut and other perennial crops, including arecanut, oil palm, agro-forestry trees and tea, especially in Asia. BSR is widely prevalent in coastal sandy soils or sandy loam soils where coconut is raised under rainfed conditions and much attention was not paid for cultural practices (Bhaskaran and Ramanathan 1984, Papa Rao and Govinda Rao 1966, Srinivasulu *et al.* 2001a, 2002a). However, the disease is not confined to any particular soil type (Bhaskaran and Ramanathan 1984).

Surveys conducted in southern states of India over the years revealed that the disease intensity varied in different states and maximum intensity of 50% was reported in Tamil Nadu. Vijayan and Natarajan (1972) conducted surveys in different districts of Tamil Nadu and reported that an average disease intensity of 6.5% in coastal coconut belt of Thanjavur district and the disease maximum reached up to 50% in Thambikottai village during the survey. Bhaskaran and Ramanathan (1984) reported that disease was more in Thanjavur district followed by Chingelput district and the incidence ranged from 0.6 to 4.9%. In some of the severely infected gardens, the disease incidence was as high as 31.4%.

Occurrence and spread of BSR in different districts of Andhra Pradesh, Karnataka and Tamil Nadu was assessed during 2010-15 (Snehalatharani *et al.* 2014a). In all the three states, the disease was found as a major threat to coconut growers. It was severe in East Godavari, West Godavari, Srikakulam, Visakhapatnam and Vijayanagaram districts of Andhra Pradesh; Hassan, Chikkamagalur, Tumkur and Shivamogga districts of Karnataka and Thanjavur, Thiruvarur and Nagapattinam districts of Tamil Nadu. East Godavari District in Andhra Pradesh recorded maximum mean per cent disease incidence of 13.82 followed by Srikakulam and West Godavari. Hassan district recorded maximum mean per cent disease incidence of BSR (7.46) followed by Chikkamagalur and Tumkur districts in Karnataka. In Tamil Nadu, Thanjavur district recorded maximum mean per cent incidence of BSR (6.5) followed by Nagapattinam and Thiruvarur.

Studies on seasonal incidence of the disease found that disease incidence was more between March and August. In general, bleeding symptoms and number of wilted palms were more during these months (Bhaskaran and Ramanathan 1984). Vijayan and Natarajan (1972) reported that more number of deaths occur during summer though the different symptoms are present throughout the year.

Generally palms succumb to BSR within two to three years of initial appearance of symptoms. But there were instances where sudden wilting occurs within six months of appearance of initial symptoms and occasionally the palms survive the initial attack and live for a number of years (Vijayan and Natarajan 1972).

Symptomology

The disease produces multiple symptoms on roots, stem and on crown region of the palm and identification of the disease is often confused with stem bleeding disease. Disease symptoms progress slowly, but usually every infected plant eventually dies. Disease develops from the roots and the first visual symptoms are visible on stem as reddish brown exudation. Peries *et al*. (1975) presented a detailed description of the symptomatology of the disease. Nambiar and Rethinam (1986) made some distinguishing characteristics that help in disease diagnosis as both Ganoderma wilt and stem bleeding diseases in coconut produces similar type of symptom such as exudation of reddish brown fluid from the stem. According to Thirumalaiswamy *et al.* (1992), palms in Thanjavur wilt, sometimes succumb without expressing external symptoms. Palms aged 10 years and older were more susceptible to the disease than younger palms.

Roots: The pathogen first infects the root system and during the very early stage of infection no external disease symptoms are clearly visible. Initially a few roots get infected and rot. Extensive rotting and discoloration of root system is a characteristic symptom of the disease and the rotting proceeds towards the bole thus, cortical tissues disintegrate and the stele turns brown. The roots are watery with a distinct alcoholic smell. The production of new roots decreases in the infected palm. In severely infected palms, more than 70 % root rotting was observed (Rethinam 1984, Bhaskaran 1986, Srinivasulu and Rao 2007).

Stem: From the roots, the infection slowly progresses up the stem leading to internal disintegration of cortical and stele tissues. Exudation of reddish brown viscous fluid from the basal portions of the stem is the first visible symptom of the disease in the affected palm. By that time, the rotting would have progressed from the bole to the basal portion of stem. Karthikeyan *et al.* (2006a) reported that the disease caused 15 to 25% damage to roots and bole below the ground level by the time external symptoms are visible. The internal tissues of the affected stem turn brown in color and rotting in the stem can be seen up to the height of the bleeding. Bleeding on the stem begins at the base and may extend up to 15 feet in severe cases (Vijayan and Natarajan 1972, Bhaskaran *et al.* 1989). Occasionally, some infected palms do not show bleeding symptoms (Thirumalaiswamy *et al.* 1992). The bark from the base of the stem peels off. Infestation of scolytid beetle, *Xyloborus perforans* and the weevil, *Diocalandra stigmaticollis* are found infesting the stem in severely infected palms. Sporophores of the fungus, *G. lucidum* appear at the base of the affected palms prior to

wilting or just after the death of the palm (Bhaskaran *et al.* 1982, Vijayan *et al.* 1973, Rethinam 1984, Bhaskaran 1986, Srinivasulu and Rao 2007).

Crown: The leaflets exhibit wilting symptoms and outer one or two whorls of leaves turn yellow. Later, they exhibit light to moderate browning followed by drooping and drying. As the disease advances, the remaining leaves also droop down in quick succession and the spindle alone remains. Vijayan and Natarajan (1972) reported that the first external symptoms are flaccidity and folding of leaflets, chlorosis and bronzing of lower whorl of leaves. Under prolonged infection, the outer leaves falloff one by one, leaving only the spindle with a few unhealthy leaves around. The spindle leaves which emerge subsequently are reduced in size and do not unfold properly. Later stem shrivels and dries up. In some cases leaves breakoff near the base along the midrib. Soft rot of bud may also set in some cases emitting bad smell. In advanced stages, all the leaves drop off leaving very thin decapitated stem (Vijayan and Natarajan 1972, Bhaskaran *et al.* 1982, Bhaskaran 1986). As the disease progresses, number of flowers, number of buttons reduces and normal development is arrested leading to button shedding. The leaves droop down resulting in hanging down of the subtended bunches. Most of the palms bear profusely, just prior to and at the time of initiation of symptoms (Vijayan and Natarajan 1972, Bhaskaran 1986 and Srinivasulu and Rao 2007).

Etiology

The genus *Ganoderma* belongs to the family Ganodermataceae which causes white rots in many woody plants by decomposing lignin as well as cellulose and related polysaccharides (Hepting 1971). *Ganoderma lucidum* on coconut was first recorded by Butler under the name *Formes lucidus* (Butler 1909). Basal stem rot of coconut in Indian subcontinent is reported to be caused by *G. lucidum* (Leys.) Karst., *G. applanatum* (Pers.) Pat. and *G. boninense*.

The aerial mycelium of *Ganoderma* is hyaline, thin walled, branched with frequent clamp connections, 1.4 to 2m in diameter, abundantly formed chlamydospores which are slightly thick-walled, terminal or intercalary, ellipsoid and sometimes in chains. 8.8-11.8 m × 3.7-5.9 m in size; cuticular cells from crustose layer are hyaline to light brown, round to irregular in shape and closely packed, presence of staghorn hyphae with projections in some isolates. Submerged mycelium is thin walled, hyphae and chlamydospores as in aerial mycelium (Sen 1973, Govindu *et al.* 1983). The fruiting body is perennial, stipitate, usually lateral, sometimes sessile, corky becoming woody later, usually 10-12 × 10-12 × 3-4 cm, but may grow up to 30 cm or more, upper surface is shining, laccate crust, ox-blood in colour and smooth. The palisade hyphae is about 40 m long and is impregnated with a dark orange varnishing substance which they secrete. Hymenial surface is whitish or creamish, turning brown later, small pores, round, 90-250 in diameter. Pore tubes are about 6-7 mm long, basidiospores are brown, thick walled, minutely verrucose, truncate at one end and 8.3-10.0 × 5.8-6.7 m in size (Bose 1930, Govindu *et al.*1983). Usually, different species of *Ganoderma* produce different feature and pathogenecity (Wong *et al*. 2012). The species identification or differentiation of *Ganoderma* is still limited; thus, this lack of information causes crucial problem for disease management. Preliminary studies on scanning electronic microscopy of *Ganoderma applanatum* and *G. lucidum* isolated from basal stem rot infected coconut could able to differentiate both the species. Spores of *G. applanatum* are single, dumbbell shaped where as that of *G. lucidum* are bundled together in round balls (Anonymous 2005).

Association of *Ganoderma lucidum* with BSR disease of coconut was reported by Vijayan and Natarajan (1972) and Bhaskaran *et al.* (1990). Pathogenicity of *Ganoderma* spp. isolated from Ganoderma wilt infected coconut palm was proved by many researchers. Both *G. applanatum* and *G. lucidum* were isolated from the diseased root bits of coconut. However, colonization of *G. lucidum* was very fast when compared to *G. applanatum*. Root rotting up to 21% was observed in palms inoculated with *G. lucidum* and only colonization up to 8 to 10 cm on either side of inoculation point was observed with *G. applanatum* (Bhaskaran *et al.* 1991, Srinivasulu *et al.* 2005). Naik *et al.* (2008) reported that disease symptoms developed in coconut seedlings after 9 to 11 months under artificial inoculation conditions. Occurrence of both the species as the causal organism of basal stem rot of coconut was reported and there was wider variation morphologically and genetically among the isolates of *Ganoderma* collected from various districts of the states (Anonymous 2014).

Host range

The genus *Ganoderma* has wide host range infecting variety of palms belonging to plantation crops, forest trees and fruit trees. More than 44 species from 34 genera of plants have been identified as potential hosts of which coconut and oil palm as the main hosts for BSR (Venkatarayan 1936). Naidu *et al.* (1966) reported that hosts belonging to 19 families, 36 genera and 48 species have been found infected with *Ganoderma* spp. Besides coconut, the fungus has been recorded on *Acacia* spp., *Acrocarpus fraxinifolius*, *Albizia* spp., *Aquillaria agallocha*, *Areca catechu*, *Bosweilia serrata, Cassia* spp., *Casuarina equisetifolia, Dalbergia* spp., *Delonix regia, Elaeis guineensis, Eucalyptus citridora, Ficus* spp., *Hevea* spp., *Jacaranda acutifolia, Lannea grandis*, *Mangifera indica*, *Melia azadiracta*, *Morus alba*, *Pinus roxburghii*, *Pleiogynium cerasiferum, Pongamia pinnata*, *Populus euramericana, Pterocarpus marsupium, Quercus semecarpifolia, Shorea robusta, Sterculia villosa, Tamarindus indica, Terminalia* spp., *Toona cibiata* and *Vitis vinifera* (Bhaskaran *et al*. 1989 and Srinivasulu and Rao 2007).

Epidemiology

BSR disease is a fatal disease affecting young and

actively bearing trees and recovery is very rare after infection (Vijayan and Natarajan 1972). Generally palms aged above 15 years alone are infected with basal stem rot disease (Bhaskaran and Ramanathan 1984). Naidu *et al.* (1966) observed incidence of BSR disease on young palms less than 10 years old as well as palms aged above 40 years.

Soil: The disease is mostly prevalent in sandy soils and where coconut gardens are raised under rainfed conditions (Bhaskaran and Ramanathan 1984, Papa Rao and Govinda Rao 1966, Srinivasulu *et al.* 2001a, 2002a). Lack of soil moisture during summer months, presence of old infected stumps in the garden, injury to roots and non-adoption of recommended cultural practices favored the disease spread. According to Peries *et al.* (1975), the disease progress rapidly in dry areas and more slowly in wet areas. In the study, the infected palms died within 6 to 30 months when the annual rainfall was 1 000 mm, whereas the others which received more than 2 000 mm annual rainfall lasted five to six years even after infection.

Soil moisture stress experienced during summer months was found to favour the spread of the disease. Ramasami *et al.* (1977) reported that presence of hard pan in the subsoil impedes root penetration and predisposes the coconut palms to the disease. Significant positive correlation was observed between soil temperature and disease and negative correlation was observed between soil moisture and disease (Bhaskaran *et al.* 1990, Karthikeyan *et al.* 2006b).

Weather: Role of weather factors such as temperature, rain fall, rainy days, relative humidity on disease development was studied. Significantly positive correlation of the disease was observed with maximum temperature, minimum temperature (Karthikeyan *et al.* 2006b) and negative correlation of the disease was observed with total rainfall, number of rainy days and relative humidity (Srinivasulu *et al.* 2001a, Karthikeyan *et al.* 2006b, Palanna *et al.* 2012). Ramapandu *et al.* (1981) reported that disease spread was more when the range of difference in relative humidity was higher and rainfall was lesser.

Disease indexing

Development of disease index for BSR helps in identification of precise disease severity and there by application of correct management measures. In coconut, indexing for assessing BSR severity was reported by Vijayan and Natarajan (1972) and Bhaskaran and Karthikeyan (1994). Vijayan and Natarajan (1972) developed indexing method based on bleeding patches on the stem and death of the palm. However, the method has a disadvantage of disease index exceeding 100 when the palm dies and there is no upper limit for disease index.

Bhaskaran and Karthikeyan (1994) refined the disease index taking into consideration the factors such as height of bleeding patches on the stem, number of leaves and reduction in leaf size. Disease Index, D.I = 23.6 + 17.7 h + 3.6 r – 0.6 l where 'h' is the height up to which bleeding has spread in the stem, 'l' is the number of functional leaves in the crown and 'r' is the score for reduction in leaf size. An index score of 15 and below was classified as mild, 15 to 40 as moderate and above 40 as severely diseased. In this method, severity does not exceed 100 and disease assessment is possible in palms that only show leaf symptoms and not on the stem.

Early detection of BSR

One of the major reasons for the devastating nature of BSR is the inability to detect the disease in early stages as it infects underground root system first apart from other factors. Management practices are most effective only when the disease is diagnosed at the early stages. A few methods were reported to be useful for early diagnosis of the disease. The methods include biochemical methods using (i) ethylenediamine-tetraacetic acid (EDTA), (ii) indicator plants such as red gram and bengal gram and (iii) molecular methods using ELISA and PCR techniques.

Biochemical methods: A colorimetric method using ethylene diamine tetra acetic acid (EDTA) was reported by Natarajan *et al.(*1986) and Karthikeyan and Bhaskaran, (2004) in which optical density of the sap from infected stem or root tissues was found to increase with increase in disease intensity. Vijayaraghavan *et al.* (1987) used transpiration rate which was significantly low in diseased palms while the stomatal diffusive resistance was slightly higher than healthy palms for early detection of the disease. Iodine-potassium iodide staining technique is the other biochemical method developed for early detection of the BSR disease.

Indicator plants: Red gram and Bengal gram plans were identified as indicator plants for BSR before visual symptom expression in coconut (Srinivasulu *et al.* 2006, Rajappan *et al.* 2011, Snehalatharani *et al.* 2014). Red gram (*Cajanus cajan L.*) plants showed wilting and longitudinal bark splitting of the stem within three months of sowing in diseased coconut palm basin. Production of sporophores at the base of red gram plants was also observed after four months (Rajappan *et al.* 2011). Bengal gram plants showed symptoms of wilting such as withering and yellowing of the older leaves followed by younger leaves within one to two months period. (Snehalatharani *et al.* 2014).

Molecular method: Currently, advance molecular techniques have been innovated with more accuracy in detection and identification of plant pathogens. The diagnostic methods used for early detection of *Ganoderma* infection in coconut are (i) ELISA based serological tests using polyclonal antibodies (PAbs) (Srinivasulu *et al.* 2003, Karthikeyan *et al.* 2007) and monoclonal antibodies (MAbs) (Rajendran *et al.* 2009, Shamala *et al.* 2006) and (ii) Polymerase chain reaction (PCR) based methods using specific primers for the pathogen (Karthikeyan *et al.* 2007; Snehalatharani *et al.* 2014).

Srinivasulu *et al.* (2003) reported simple serological techniques such as slide agglutination, glass capillary tube tests and ELISA as useful techniques for early detection of BSR in coconut. Karthikeyan *et al.* (2007) raised PAbs against mycelial basidiocarp and specific proteins of

Ganoderma which could able to detect the pathogen in diseased coconut root tissues before symptom expression at the antiserum dilution of 1: 1 000 for mycelial protein, 1 : 700 for *Ganoderma* specific protein and 1 : 3 000 for basidiocarp protein. Rajendran *et al.* (2009) raised monospecific antibodies for 62kDa protein of *Ganoderma* and found positive reactions for infected samples. Shamala *et al.* (2006) also attempted generation of monoclonal antibodies against *Ganoderma boninense* of oil palm. However, the antisera based methods showed cross-reactions with saprophytic fungi and other basidiomycetes fungi in coconut (Karthikeyan *et al.* 2007) and in oil palm (Shamala *et al.* 2006) to some extent.

In PCR based methods, primers Gan1 (5'-TTG ACT GGG TTG TAG CTG-3') and Gan2 (5'-GCG TTA CAT CGC AAT ACA-3') which could amplify 167bp region from internal transcribed spacer region of rDNA were used for detection of BSR in coconut (Karthikeyan *et al.* 2008, Snehalatharani *et al.* 2014b). The primers could able to amplify expected band size in the presence of the pathogen. In addition to Gan1 and Gan2 primer set, Mandal *et al.* (2014) used *Gan* ET (5'-GAG TTG TCC CAA TAA C-3') and ITS3 (5'-GCA TCG ATG AAG AAC GCA GC-3') primers for the detection of BSR in oil palm. Both the sets of primers detected *Ganoderma* infection accurately in oil palm roots of infected palms. Karthikeyan *et al.* (2007) reported that a combination of polyclonal antibodies and PCR based methods were highly reliable, rapid and sensitive in early detection of BSR in coconut.

Pathogen diversity

Understanding the biology and the existing pathogen diversity is the first step in successful management of the disease. Diversity analysis and grouping of any organism are through cultural, morphological and molecular based studies which have their own advantages and disadvantages. In *Ganoderma*, the morphological characteristics have limitations like absence of basidiocarps during certain times of the year, morphological plasticity and presence of cryptic species. Zheng *et al.* (2007) reported that the identification of *Ganoderma* species is very difficult because of environmental influence, variability, inter hybridization and morphological propensity. Isozyme analysis, Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Internal Transcribed Spacers (ITS), Random amplified Polymorphic DNA (RAPD), Mitochondrial DNA analysis are the various molecular techniques being used for analyzing the genetic diversity in basidiomycetes throughout the world. In India, grouping and diversity analysis of *Ganoderma* spp. in coconut were carried out through Isozyme analysis (Snehalatharani *et al.* 2012), RAPD and RAMS PCR analysis (Lakshmi *et al.* 2010; Anonymous 2014).

Grouping and diversity analysis studies based on molecular methods reported wide genetic diversity within and between isolates of *Ganoderma* from coconut and oil palm (Lakshmi *et al*. 2010, Zakaria *et al*. 2005, Snehalatharani *et al.* 2012, Rakib *et al.* 2014, Anonymous 2014). Lakshmi *et al.* (2010) studied genetic diversity of 12 *Ganoderma* isolates using RAPD and RAMS PCR analysis. RAPD PCR detected 0 – 75% of genetic similarity among the isolates and RAMS PCR detected 0 and 72% of genetic similarity among the same isolates. Genetic relatedness within and between *Ganoderma boninense* isolates of oil palm and *Ganoderma* spp. from coconut from different locations of Malaysia was assessed by RAPD & RAMS PCR (Zakaria *et al.* 2005). Both analysis showed variations of banding patterns within and between the isolates from oil palm and coconut stumps, indicating that they were genetically heterogeneous. Important finding from the study was *G. boninense* from oil palm and *Ganoderma* spp. from coconut stumps did not cluster separately and were clustered together. This indicates that both groups of *Ganoderma* are closely related.

Esterase, catalase, peroxidase and malate dehydrogenase enzyme profiles of 24 *Ganoderma* isolates from coconut were analyzed by Snehalatharani *et al.* (2012). The malate dehydrogenase profile grouped 20 of the 24 isolates into two categories (three loci or five loci) except four isolates which showed 2 and 4 loci. Grouping of the isolates based on malate dehydrogenase profile was similar to that of the RAPD PCR profile generated earlier with 12 isolates of *Ganoderma* from coconut by Lakshmi *et al.* (2010).

Rakib *et al*. (2014) studied the genetic and morphological diversity of *Ganoderma* species isolated from upper stem rot (USR) and basal stem rot (BSR) of infected oil palm through multiplex PCR. The diversity studies revealed association of three pathogenic *Ganoderma* species (*G. zonatum*, *G. boninense* and *G. miniatocinctum*) in 46 samples. *Ganoderma zonatum* was the dominant species (71.7%), followed by *G. boninense* (26.1%) and *G. miniatocinctum* (2.2%).

RAPD PCR based clustering of 35 *Ganoderma* isolates of coconut from Andhra Pradesh, Karnataka and Tamil Nadu showed that the genetic similarity among the isolates ranged from 0 to 88.4% (Anonymous 2014). The grouping obtained through RAPD – PCR goes hand in hand with that of Isozyme analysis and grouping based on pathogenic virulence studies. High degree of variation among the Ganoderma isolated may be attributed to the geographical differences in isolate collection, or wide genetic base within species or from closely related species. Rees *et al.* (2012) through RAMS PCR studies demonstrated that isolate diversity of *G. boninense* from oil palm was as great within a plantation as between plantations. Basidiospores play an important role in spread and genetic variability of *G. boninense* in oil palm.

As the degree of virulence of white rot fungi is governed by laccase enzyme activity, assessing laccase activity of 25 *Ganoderma* isolates belonging to different host species was attempted by Rajendran *et al.* (2008). The partial sequence of laccase gene from highly virulent isolate was cloned and sequenced which showed high homology with laccase genes of other basidiomycetes.

Disease management

The disease is considered as most destructive as it escapes early symptoms and having various resistant stages such as resistant mycelium, chlamydospores, basidiospores and pseudosclerotia. Researchers across the country conducted various experiments on screening for disease resistance and to find out effective management measures. None of the germ plasm screened against the disease showed resistance to the disease. Several management trials were conducted using cultural, nutritional, biological and chemical measures against the disease. However, results of the disease management trials depend upon soil condition, age of the crop, stage of disease development and weather parameters of that particular area. Although it is impossible to manage a field free of pathogen (Sanderson *et al.* 2000) considerable reduction of the disease can be achieved through a proper integrated disease management system.

Screening for disease resistance: Forty four coconut varieties/ hybrids were screened for identification of resistance against BSR from 1984 under AICRP on Palms. However, none of the germplasm showed resistance to the pathogen. The varieties or hybrids screened under the programme are San Ramon OP, San Ramon SP, San Ramon IC, B.S. Islands OP, B.S. Islands SP, B.S. Islands IC, Java OP, Java SP, Java IC, S.S.Green OP, S.S.Green SP, S.S.Green IC, Guam OP, Guam SP, Guam IC, St. Vincent OP, St. Vincent SP, St. Vincent IC, L.O.OP, L.O. SP, L.O. IC, TXD (WCT × COD), DXT (COD × WCT), TXD (ECT × GB), West Coast Tall, East Coast Tall, GB × PO, GB × Fiji, GB × LO, Gangabondam, Andaman Ordinary, Chowgat Orange Dwarf, Malayan Yellow Dwarf, Philippines Ordinary, Cochin China, VHC – 1, VHC–2, Java, Kera Ganga, Laksha Ganga, Chandra Laksha, Godavari Ganga, Chandra Sankara (Srinivasulu *et al.* 2005).

Cultural and nutritional management: Various cultural and nutritional management measures were studied for containing severity of the disease. The methods include digging isolation trenches, removal and burning of dead infected plant material, avoiding ploughing and flood irrigation, growing intercrops etc. Butler (1926) and Venkatarayan (1936) reported that digging of isolation trenches which separates diseased and healthy palms for the management of the disease.

Destruction of dead and decayed plant material was suggested by many researchers (Bhaskaran *et al.* 1990, Bhaskaran *et al.* 1994). Removal of the affected area of the diseased palm and application of 10% copper sulphate was recommended by Bhaskaran *et al.* (1990). Burning of brick kilns in sites of disease incidence or application of sulphur dust all over the diseased area appears to have the same effect as destruction of infected plant material (Bhaskaran *et al.*1994).

Bhaskaran *et al.* (1989) reported that irrigation with fertilizer application increased disease intensity, whereas irrigation combined with Bordeaux mixture drenching checked the disease intensity considerably. Nambiar and Rawther (1993) reported that ploughing and flood irrigation should be avoided to prevent the spread of infective propagules.

Karthikeyan and Bhaskaran (2001) reported that growing intercrops such as *Desmodium tortuosum, Calopogonium mucunoides, Tephrosia purpurea, Crotalaria juncea, Curcuma domestica* [*Curcuma longa*] and *Musa* sp. reduced disease incidence, increased soil antagonistic microorganisms like *Trichoderma* and bio fertilizers, viz. phosphobacteria, *Azotobacter* and *Azospirillum*. Among the intercrops, banana was very effective in containing the disease.

Macro and micronutrients also influence the disease progress in coconut. Application of 350, 250 and 450g of N, P_2O_5 and K_2O, respectively, per palm per year showed low disease index while higher doses of fertilizers increased disease intensity (Bhaskaran *et al.* 1978, Bhaskaran and Ramanathan 1983). Application of Manganese sulphate (227g/ palm/ year), Zinc sulphate (2%), sulphur and lime reduced disease intensity where as application of molybdenum increased disease intensity (Jaganathan and Ramasami, 1975, Bhaskaran *et al.* 1985, Venkatarayan 1936 and Srinivasulu *et al.* 2002b). Papa Rao and Govinda Rao (1966) reported that in the plots treated with sulphur dust, the rate of disease spread was slower initially and faster after six months than in check. However, Sindha Mathur *et al*. (1983) could not observe any direct effect of micronutrients on the disease.

Irrigation, application of farm yard manure combined with burying coconut husk around the diseased palms controlled the disease (Vijayan and Natrajan 1972, 1975; Bhaskaran *et al.* 1978, Suriachandraselvan and Bhaskaran 1999). Application of 50 kg farmyard manure or green leaves or 5 kg neem cake or 300 kg tank silt per palm per year arrested disease progress (Vijayan and Natarajan 1975). Neem cake alone or in combination with drenching of 1% Bordeaux mixture was most effective in reducing the disease intensity of BSR (Bhaskaran and Ramanathan 1983).

Chemical management: Among the several chemicals tried for the control of the disease, Bordeaux mixture, Heptachlor dust and Copper oxy chloride along with BHC controlled the disease to certain extent if applied in earlier stages of infection (Vijayan and Natarajan 1972). Bordeaux mixture (1%), Aureofungin-sol (0.2%) chemicals alone or in combination were reported as effective chemicals by several workers (Bhaskaran and Ramanathan 1982, Bhaskaran *et al.* 1984). Drenching with 10 litre of Benomyl (0.1%) was reported by Kolandaisamy and Arjunan (1977). *In vitro* inhibitory effect of Tridemorph (500 ppm) was reported by Anbalagan and Shanmugam (1984). Sindha Mathur and Balasubramaniam (1987) reported that soil drenching with 0.1% IBP, carboxin, tridemorph or 0.05% carbendazim in combination with 5 kg neem cake per palm reduced disease intensity. Field trials at Palghat (Kerala), Veppankulam (Tamil Nadu) and Andhra Pradesh reported that tridemorph and Aureofungin-sol in combination with neem cake reduced disease intensity. Other studies reported that Aureofungin-sol (2 g) + 1% Bordeaux mixture (40 litre

) + neem cake (5 kg) checked further spread of the disease. The lowest BSR index was obtained by the application of Tridemorph root feeding (2%) + soil drenching (0.3%) followed by Hexaconazole root feeding (l%)+ soil drenching (0.2%), soil drenching with Tridemorph (0.3%) and Hexaconazole (0.2%) compared to root feeding alone (Naik 2001). Fungicides viz., Bordeaux mixture, Triademifon, Tridemorph, Bitertenol, Copper Oxy Chloride, Hexaconazole were found to inhibit *G. applanatum* and *G. lucidum* under *in vitro* conditions and were also found inhibitory to *Trichoderma viride* (Srinivasulu *et al.* 2002). According to Thirumalaiswamy *et al*. (1992), treatments with fungicides to be taken up by roots are effective only in the early stages of the disease.

Biological management: Antagonistic micro organisms: Trichoderma species (*T. harzianum, T. viride, T. hamatum, T. longibrachiatum, T. virens, T. polysporum), Pseudomonas fluorescens, Bacillus subtilus* are the antagonistic microorganisms reported against *Ganoderma* spp. in India. Papa Rao *et al.* (1975) reported that *G. lucidum* fail to grow under *in vitro* conditions on unsterilized coconut root bits and also on sterilized roots which were contaminated with species of *Trichoderma, Aspergillus* and *Rhizopus*. Application of 5 to 10 kg neem cake per palm per year encouraged saprophytic soil microflora especially *Trichoderma* in coconut basins and was effective in the control of BSR (Gunasekaran *et al.* 1986, Bhaskaran *et al.* 1988, Bhaskaran 1990). Among the *Trichoderma* species, *T. harzianum, T. viride, T. hamatum, T. longibrachiatum, T. virens, T. polysporum* are reported to be having antagonistic activity against *G. applanatum* and *G. lucidum* (Gunasekaran *et al.* 1986, Bhaskaran *et al.* 1988 Bhaskaran *et al.* 1990, Srinivasulu *et al.* 2002b, 2004a, b). Studies on suitable substrate for mass multiplication of *Trichoderma* spp. found that rice bran, coir dust, sugarcane bagasse, and sorghum grain as suitable substrates; however, neem cake is the most efficient (Bhaskaran *et al.* 1988, Srinivasulu *et al.* 2005). Application of 50 g of *T. viride* along with 5 kg of neem cake per palm per year controlled the linear spread of BSR within a period of four months (Srinivasulu *et al.* 2001b). Talc based formulations of *Trichoderma* and *Pseudomonas* in combination with neem cake were on par with chemical treatment i.e root feeding of 1% hexaconazole in containing the disease (Srinivasulu *et al.* 2004a, 2006; Naik *et al.* 2008). Palanna *et al.* (2013) reported that among the 17 biocontrol agents screened, native *Trichoderma* sp. (V2) recorded 81% reduction over control in dual culture studies against *G. applanatum*.

Pseudomonas fluorescens was reported to be having antagonistic activity against *Ganoderma* spp. under *in vitro* conditions (Srinivasulu *et al.* 2004b, Karthikeyan *et al.* 2005, George *et al.* 2012). George *et al.* (2012) screened 156 fluorescent pseudomonads against *G. applanatum* and found that 8% of the total fluorescent pseudomonads showed antagonism towards *G. applanatum* (inhibition ranging from 39 to 73%). Rajendran *et al.* (2010) suggested endophytic *Bacillus subtilis* as effective bio control agent for management of BSR. George *et al.* (2011) isolated 327 heat resistant, endospore producing bacilli from the rhizospheric soil and roots of coconut growing in Kerala, Tamil Nadu, Karnataka, Andhra Pradesh and Maharashtra and tested against *Ganoderma applanatum*. More than 90 % of the isolates were found to effectively inhibit the mycelial growth of *G. applanatum*, with percentage inhibition ranging from 44 to 91.

In the field studies talc formulation of *T. hamatum* (50 g/palm) along with 5 kg neem cake was effective when compared to *T. harzianum* and *T. viride* (Srinivasulu *et al.* 2004a, 2005). Karthikeyan *et al.* (2005) also suggested similar effect of *Trichoderma* and *Pseudomonas* bio agents against basal stem rot however, the quantity of the talc formulation was higher (200 g/ plant). Surulirajan *et al.* (2014) reported that application of talc formulation of *T. viride* (200 g/palm/year), TNAU microbial consortia and neem cake gave equal effect with that of soil drenching of Bordeaux mixture and root feeding of Tridemorph (2 ml in 100 ml of water). Karthikeyan *et al.* (2005) suggested that frequency of application of bioagents should be at three month interval based on the rhizosphere populations of the bioagents.

In the studies to unveil antagonistic nature of *Trichoderma*, Srinivasulu *et al.* (2004a) reported that the species were found to be very effective in producing volatile and non volatile metabolites against *Ganoderma* spp. Scanning electron microscopy studies of antagonism of *Trichoderma* spp. against *G. applanatum* and *G. lucidum* clearly showed mycoparasitism of *Trichoderma* spp. (Anonymous 2005). Karthikeyan *et al.* (2006) reported induction of phenolics and defense related enzymes in coconut roots treated with biocontrol agents. Soil application of these biocontrol formulations in combination with chitin induced significant increase in activities of peroxidise, polyphenol oxidase, phenylalanine ammonia lyase, chitinase and β -1,3 glucanase.

Plant extracts: Various studies reported that plant extracts of neem, banana, Tephrosia, garlic, Pongamia, *Prosopis julifera*, Glyricidia, *Eichhornia crassipes* were effective against *Ganoderma* spp. under *in vitro* conditions. In field conditions, plant extracts of *Pongamia glabra*, *Azadirachta indica* and *Prosopis julifera* recorded lesser disease index compared to the control. Bhaskaran *et al.* (1988) reported suppressive nature of neem, banana rhizome extract and *Tephrosia purpurea* root extract (100%, 86% and 54% inhibition, respectively) against *Ganoderma lucidum.* After screening of 19 weed and plant extracts against *Ganoderma* spp. under *in vitro* conditions, Srinivasulu *et al.* (2005) found that 10% of garlic extract completely inhibited the growth of *Ganoderma* spp. Karunanithi *et al.* (2007) evaluated 29 plant products under *in vitro* and *in vivo* conditions for the management of BSR. Among them, leaf extracts of *Pongamia glabra*, *Azadirachta indica* and *Prosopis julifera* at 10% concentration were found effective in suppressing the mycelial growth of *Ganoderma lucidum in vitro*. In field conditions, these

plant products recorded lesser disease index of BSR compared to the control. Palanna *et al.* (2013) reported in *vitro* antifungal nature of *Glyricidia* plant extract against *Ganoderma applanatum*. Deepatharshini and Elango (2015) studied the antifungal activity of *Eichhorinia crassipes* against *Ganoderma lucidum* at two concentrations (150 and 300 mg/ml). Plant extract at 300 mg/ml was found more effective.

Integrated disease management: As the disease is a constant threat to coconut farmers of southern states of India, various authors attempted integrated disease management approaches to control the disease.

Soil drenching with 1% Bordeaux mixture (40 l/palm) and application of neem cake (5 kg/palm) in basins followed by root feeding with Tridemorph (6 ml/palm) was found more effective in reducing the disease index of BSR in Andhra Pradesh (Srinivasulu *et al.* 2005). Residue analysis for the rate of accumulation of Tridemorph @ 2, 4, 6 ml/palm in coconut water and copra after root feeding showed that the residues were at lower than tolerance limits. Karthikeyan *et al.* (2006) found that the combination of basin method of irrigation, soil application of neem cake (5 kg/palm), soil application of talc formulations of *T. viride* and *P. fluorescens* (200 g each/palm) and root feeding of tridemorph 2% (100 ml palm at quarterly intervals) gave effective control of vertical spread of pathogen with a reduction of 92.1% over control and horizontal spread with a reduction of 45.5% over control.

Rajappan and Vaithilingam (2009) reported integrated disease management system (IMS) consisting of cultural (basin irrigation, application of the recommended fertilizer rates, application of 50 kg farmyard manure/plant, basin management with 50 g sunnhemp and *in situ* incorporation, and intercropping with banana in 2 rows within 2 rows of coconut), biological (application of *Trichoderma viride* and *Pseudomonas fluorescens* at 200 g plant each) and chemical (application of tridemorph or hexaconazole at 200 ml/100 ml of water at quarterly intervals) control systems were effective against basal stem rot disease.

Palanna *et al.* (2009) found that the disease spread was less (70.31% reduction over control) with root feeding of Hexaconazole (1%) at quarterly intervals along with soil application of 5 kg neem cake and 50 g of *Trichoderma harzianum* per palm at half yearly interval. Root feeding of tridemorph (2%) along with application of 5 kg neem cake/palm/year and root feeding of hexaconazole (1%) combined with application of 5 kg neem cake/palm/year also reported less disease spread (64.02 and 56.93% reduction over control, respectively).

Large scale demonstrations of *Trichoderma* based bio management for BSR was conducted at five locations (Kalavacharla, *P. Gannavaram* and *G. Pedapudi* villages of East Godavari District; Jagati and Borivanka villages of Srikakulam district) in Andhra Pradesh at the rate of 50 acre area per each demonstration. Application of *T. viride* (50 g) along with neem cake (5 kg) was assessed from March 2012 to August 2013 (Snehalatharani and Maheswarappa 2015). The demonstrations recorded significant disease reduction, increased leaf number and nut yield in implemented villages. The treated palms showed dried symptoms as well as reduced linear disease spread on the trunk.

In an another study, bio control combination of 50 g of *T. viride* and 5 kg of neem cake per diseased palm per year along with integrated disease management measures against basal stem rot disease was demonstrated in an area of 5 acre at two demonstration sites Antarvedi and Keasanapalli of East Godavari District of Andhra Pradesh (Snehalatharani *et al.* 2016). These demonstrations also recorded significant disease reduction, increased leaf number and nut yield in implemented villages. The fields recorded almost doubled yield after 15 months of treatment imposition and reisolation studies confirmed the presence of *T. viride* in the implemented gardens.

In the absence of resistant germplasm (Srinivasulu *et al.* 2005), integrated disease management measures provide promising alternate approach for managing basal stem rot disease of coconut. Investigations made by various researchers across the country on cultural, biological and chemical management led to the development of effective integrated management measures and assures that basal stem rot disease of coconut can be manageable. The measures include drip or basin method of irrigation, frequent watering or irrigation especially during summer months, avoiding injury or damage to roots, raising and ploughing in situ green manure crops, uprooting and destruction of diseased and dead palms along with the roots, isolation of diseased palms from healthy palms by digging isolation trenches of 1 m depth and 0.5 m width, raising of indicator plants for early detection, application of 50 g of *T. viride* in combination of 5 kg of neem cake to the diseased palms once in every year, application of the above said mixture at the rate of 1 kg to all the healthy palms in the field and opting for root feeding of chemical fungicides Hexaconazole (1%) and Tridemorph (2%).

Basal stem rot disease of coconut is one of the major reasons for reduced yields of coconut in southern states. The disease is reported to be caused by *G. lucidum* (Leys.) Karst., *G. applanatum* (Pers.) Pat. and *G. boninense* in Indian subcontinent. Its occurrence and distribution in major coconut growing states revealed that the disease is not confined to any soil type, however is more prevalent in lighter soils. High degree of variation was observed between the pathogen isolates through morphological, cultural and molecular studies. Even though early disease escapes detection, the disease is found manageable with continuous monitoring and biocontrol based integrated disease management measures. Recent developments in early detection involving indicator plants, protein and DNA based studies found promising in detecting the disease at early stage. However, thorough research is needed in molecular identification and differentiation of the pathogen species as confusion in identification process leads to inefficient disease management. Further, identification of diversity existing between and within *Ganoderma* species in coconut assists

in developing early detection molecular markers. Continuous research for biocontrol agents and testing their on farm efficacy helps in management of this difficult soil borne pathogen in coconut.

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