



Occurrence of algal leaf spot on longan (*Dimocarpus longan*) caused by *Cephaleuros virescens* in India

VINOD KUMAR¹, AJIT KUMAR DUBEDI ANAL², ALOK KUMAR GUPTA³ and VISHAL NATH⁴

ICAR-National Research Centre on Litchi, Muzaffarpur, Bihar 842 002, India

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ABSTRACT

Longan (*Dimocarpus longan* Lour.) is an evergreen subtropical fruit tree species of Sapindaceae family with a long cultivation tradition in South East Asia. Between July to September of 2014 and 2015, leaves of longan trees were found infected by green algae at experimental farm of National Research Centre on Litchi, Muzaffarpur, Bihar (India). The symptoms of the disease appeared on the upper leaf surface as dark gray to reddish-rust coloured lesions, slightly raised with a velvet-like appearance. The disease incidence among leaves was 28.4-57.3% while percent disease severity index was 13.3 to 20.2. The algal thalli were isolated and cultured successfully on potato dextrose agar and Bold's basal medium but there was no development of sporangiophores and sporangia in culture. In pathogenicity test, inoculums either from naturally infected leaves or from pure culture of algal filaments could not reproduce typical symptom of the disease even up to three months of inoculation. Based on the leaf symptom and morphological characteristics, the pathogen was identified as *Cephaleuros virescens* Kunze. To our knowledge, this is the first report of leaf spots caused by *Cephaleuros virescens* on longan trees in India.

Key words: Algal leaf spot, *Cephaleuros virescens*, Green algae, Longan

The longan (*Dimocarpus longan* Lour., Family: Sapindaceae) is a subtropical woody perennial fruit tree cultivated in Southeast Asia, including southern China, Vietnam, Thailand, Taiwan, Cambodia, Laos, Myanmar, Queensland in Australia, Florida in the United States and northern India. World production of longan was more than 2500 million tonnes in 2010 (FAO 2011). Longan is currently grown on a smaller scale in India but there is growing interest among farmers for its cultivation. Since, the fruit is similar to litchi but matures later; the longan can be useful for extending the season for fruit of this type. Longans are consumed fresh or frozen for later consumption. The fruit is a good source of potassium and is low in calories. It is a great source of ellagic and gallic acids, which are potent anti-oxidants. So far in India, there had been no major disease problems in longan. However, during July to September of 2014 and 2015, numerous leaf spots or lesions on upper leaf surface were observed that subsequently resulted in necrosis of majority of leaf tissues. Such symptoms are often caused by parasitic green algae of the genus *Cephaleuros* on many woody trees (Brooks *et al.* 2015) mostly during high humidity and warm rainy weather. This leaf spot was noticeable on all

the nine longan germplasm (Lgc-01 to Lgc-09) at National Research Centre on Litchi (NRCL) experimental farm. Since, the disease occurred during fruiting season, it affected the yield and quality of fruits. Therefore, the objectives of the present investigation were to study symptomatology, assess extent of the disease on trees, and to identify the causal agent of the disease, its pathogenicity, morphological and microscopic characteristics.

MATERIALS AND METHODS

Diseased leaf specimens were collected from the experimental farm of NRCL, Muzaffarpur (26° 05' 43" N, 85° 26' 39" E, 47 m asl) in Bihar state of India. Symptoms were observed visually as well under a stereomicroscope. Slides were prepared by using sterile distilled water as mounting medium, removing the mycelium and algal structures found on the infected tissue (Vasconcelos *et al.* 2016). Images of micro-morphological structures were captured under bright field of a Nikon Fluorescence Microscope (Eclipse Ti-5) having photographic attachment (DS-Ri1). Dimensions of algal structures (sporangiophore and sporangia) were measured by using the 'NIS-Elements AR software', obtaining the average of 20 measurements for each structure and the range of the values were noted. The voucher specimens were preserved and accessioned in the herbarium of Crop Protection Unit, ICAR-National Research Centre on Litchi, Muzaffarpur, Bihar.

Algal cultures were obtained based on the methods of Suto and Ohtani (2011). Leaves with fresh thalli were

¹Senior Scientist (vinod3kiari@yahoo.co.in, vinod.kumar11@icar.gov.in), ²Young Professional (ajit_014micro@rediffmail.com), ³Scientist (alokguptabhu@gmail.com), ⁴Director (vishalnath66@rediffmail.com).

washed under running water for five minutes and soaked in water for one hour, wiped with cotton wool, dipped in 70% ethanol for 60 seconds, and rinsed with sterile water three times. After that, small thalli (2-3 mm) were removed with a sterile razor blade and placed on potato dextrose agar (PDA) and Bold's basal medium (BBM; Bischoff and Bold 1963, Andersen 2005), and incubated at 25 °C in a light: dark cycle of 12:12 hours. Macroscopic features of lesions and algal thalli were observed under a stereoscopic microscope. Microscopic features of thalli, filaments, and reproductive organs were observed under bright field microscope. Pathogenicity test was carried out with pure culture (filaments of thalli) as well as with natural inoculum obtained from infected leaves of orchard tree. Inoculum were prepared in sterile distilled water by scrapping thalli from 6-day old cultures growing on BBM. For preparing natural inoculum, the symptomatic leaves from orchard tree were collected, washed under running water for five minutes and soaked in water for one hour followed by wiping with cotton wool, dipping in 70% ethanol, and rinsed with sterile water. After that, some lesions from the surface of these leaves were scrapped, put in distilled water in a conical flask and vortexed. Inoculations were done on healthy mature leaves of glasshouse grown 2-year old seedlings, and also on leaves of an orchard tree. Leaves to be inoculated were first wiped thoroughly with sterile water and then inoculum were scrubbed over the leaf surface. After inoculation, the plants/leaves were covered with thin polybag having few prick-holes for aeration, and were firmly fixed with a rubber band.

Incidence and severity of the disease on orchard trees were assessed during August 2015. Four trees in each block were sampled and per tree randomly 10 twigs (small branch or shoot of a tree having approximately 100-125 leaves) were observed for disease lesions on leaves from all the four direction. Thus based on observation of 40 twigs, 'percent infected twigs' on tree was calculated. Further, four twigs were randomly observed and infected leaflets were counted to compute 'percent infected leaflets'. Percent disease severity index (PDI) on leaflets was assessed using a 5-point scale (<10% leaf area infected -1; 10-20% leaf area infected - 2; 20-30% leaf area infected -3; 30-40%

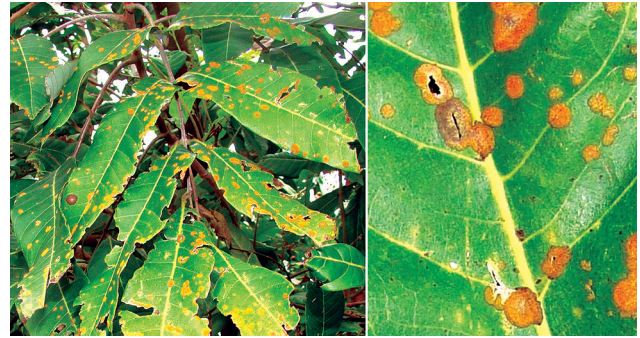


Fig 1 Symptoms on leaves of a tree (Left) and close-up view of lesions (Right).

leaf area infected - 4; >40% leaf area infected). PDI was calculated using the formula,

$$PDI = \frac{\sum (\text{Severity grade} \times \text{no. of leaflets}) \times 100}{(\text{Total no. of leaflets observed} \times \text{maximum grade})}$$

Analysis of variance (ANOVA) in data on disease incidence and severity was conducted with SAS® 9. 2 statistical software using a completely randomized block design. The least significant differences (LSDs) between means at 5% probability (P=0. 05) and the standard error (SE) of means were computed.

RESULTS AND DISCUSSION

Symptoms and occurrence of disease: The symptoms of the disease appeared as dark gray to reddish-rust coloured spots or lesions slightly raised with a velvet-like appearance on the upper leaf surface (Fig 1) that subsequently resulted in necrosis of majority of leaf tissues. The symptoms were more prevalent on older leaves, in the lower foliage of the trees. In severe infections, leaf drop and stem die back occurred. No lesions were observed on fruits. It was evident that thalli grow subcuticularly on the upper leaf surface. They were more or less circular, raised disk like, 1-8 mm (mostly 3-4 mm) in diameter and olive in colour. Algal thalli were observed in all the leaf lesions under stereobinocular microscope. No thalli were found on the lower leaf surface.

All the trees in orchard were found infected. Data revealed that percent infected twigs in trees were in the range of 20.0-37.5 while percent infected leaflet was 28.4-57.4

Table 1 Incidence and severity of algal leaf spot on longan trees at NRCL Experimental Farm, Muzaffapur (India) in August 2015

Germplasm block	Percent infected twig	Percent infected leaflet	Percent distribution of leaflets in different categories of severity			Percent disease severity index
			< 10%	10-20%	>20%	
I	35.0	40.7	69.2	16.7	14.0	16.1
II	25.0	35.7	64.4	15.8	19.8	17.3
III	22.5	28.4	48.2	31.7	19.3	18.9
IV	37.5	57.4	45.2	27.6	27.3	20.2
V	30.0	34.9	83.5	8.3	8.2	13.9
VI	20.0	46.3	74.3	12.6	13.1	15.4
VII	27.5	40.8	82.9	14.3	2.7	13.3
SEM±	0.7	1.1				0.4
LSD (P=0. 05)	2.8	3.2	-	-	-	1.2

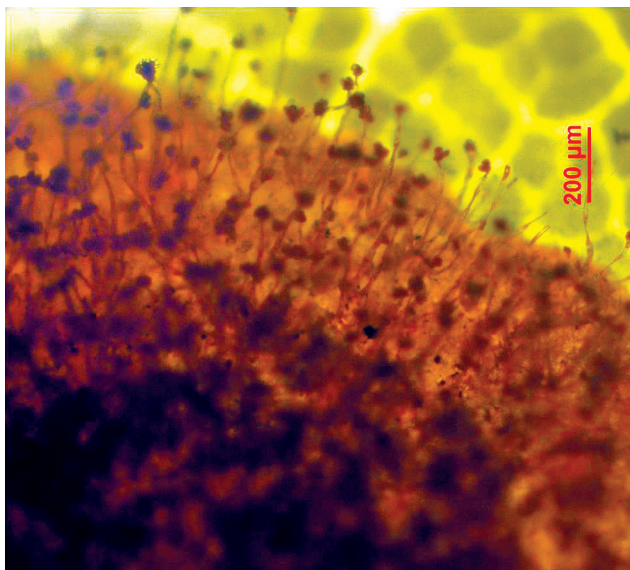


Fig 2 Lesions having tuft of sporangiophores and sporangia as seen under stereobinocular microscope.

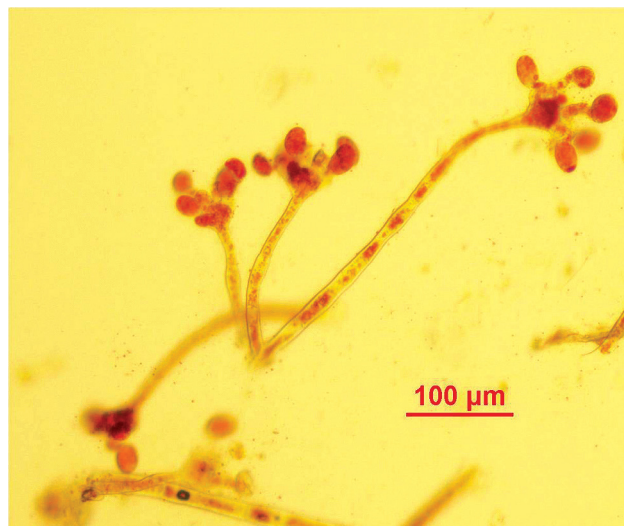


Fig 3 Photomicrograph of *Cephaleuros virescens*, the conspicuous head with sporangia is subtended by a long and narrow sporangiophore.

(Table 1). Though the PDI ranged from 13.3 to 20.2, the percent distribution of infected leaflets in different severity categories indicated that lesser number of leaves had more than 20% of area damaged by lesions caused by algal thalli. The disease was found prevalent during July to September when maximum temperature was 31.7-35.4 °C and minimum was 24.6-26.7 °C. Maximum humidity during the period was 82.6-93.6% while minimum was 39.7-59.7%. It was most prevalent during high humidity, warm and rainy weather. The pathogens reproduced and survived in spots on leaves or stems and in fallen plant host debris.

Isolation and characterization of the pathogen: The algal thalli were isolated and cultured successfully on PDA and BBM but there was no development of sporangiophores and sporangia in culture. Characteristics of the algal structures were hence studied by preparing temporary

mount from leaf lesions. Head cells were produced at the termini of sporangiophores (Fig. 2–5). The dimensions of sporangiophores were (length × width) 116.0-309.5 × 10.7-15.3 μm. Head cells produced 4 to 6 sporangiate-laterals (mostly five), zoosporangia and their suffultory cells. Mature zoosporangia were globular in shape and measured (length × width) 28.5-32.3 × 19.4-22.1 μm. Sporangium produced ellipsoidal to broadly fusiform quadriflagellate zoospores. In our collection, mature gametangia were not observed.

Based on the leaf symptom and morphological characteristics, the pathogen was identified as *Cephaleuros virescens* Kunze of the family Trentepohliaceae, order Trentepohliales and the division Chlorophyta. The morphological characteristics of the pathogen largely corroborated to the description by Thompson and Wujek (1997) and Suto and Ohtani (2009). Dimensions of microscopic structures are often influenced by host plants,

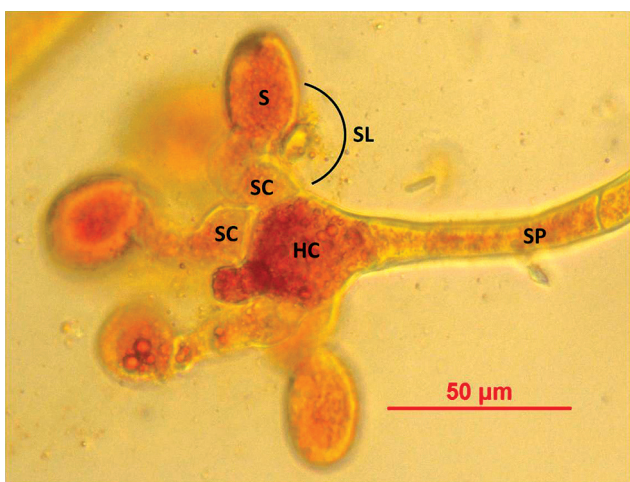


Fig 4 Photomicrograph of sporangiophores and sporangia of *Cephaleuros virescens*, SP=sporangiophore; HC=head cell; SC= suffultory cell; S=sporangium. The suffultory cell and sporangium together form the sporangiate-lateral (SL).



Fig 5 A sporangium of *Cephaleuros virescens*.

collecting seasons, and environmental conditions (Suto *et al.* 2014). Pathogenicity test was negative and typical symptoms could not be produced even up to three months of inoculation in both the inoculum types, either filaments of algal thalli from pure culture of *C. virescens* or with natural inoculum prepared from leaf lesions. Pathogenicity tests on other hosts also have never been demonstrated successfully, nor have zoospores been produced in culture (Holcomb *et al.* 1998).

Green algae in the genus *Cephaleuros* are known to be parasitic on several woody plants in tropics and subtropics such as tea (*Camellia sinensis*), avocado (*Persea americana*), breadfruit (*Artocarpus altilis*), carambola (*Averrhoa carambola*), some citrus (*Citrus* spp.), durian (*Durio zibethinus*), longan (*D. longan*), litchi (*Litchi chinensis*), mango (*Mangifera indica*), mangosteen (*Garcinia mangostana*) and rambutan (*Nephelium lappaceum*). Signs of the disease are often found on the leaf surface in the form of burnt-orange to brown spots (Nelson 2008). Leaf tissues are colonized beneath the epidermis by algal filaments, but host cells are not penetrated (Chapman and Henk 1985). In India, *C. virescens* commonly infect mango and litchi leaves. Prevalence of the disease on tea plantation in India (Ramya *et al.* 2013) and Japan (Ezuka and Ando 1994) has also been reported. Leaf spot caused by *C. virescens* on longan in Puerto Rico was reported by Ferwerda (2002), on Para rubber (*Hevea brasiliensis*) in Thailand by Pitaloka *et al.* (2015) and on rambutan in Thailand by Sunpapao *et al.* (2016). Our research indicates that longan is a new host in India for *C. virescens*. Although the incidence and severity appears to be small on current plantings, this might become a serious problem as this crop becomes better known and its cultivation increases in the country. Therefore, it is suggested to monitor trees for the algal spots and to apply control measures if it becomes necessary.

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