

## Defoliation and die-back emerging threat to Cashew cultivation in the Konkan region of Maharashtra

### Abstract:

Cashew is a million-dollar crop, and it is the second most important cash crop in the Konkan region of Maharashtra after mango. Till recently, the cashew plantations in the Konkan region were free from destructive diseases leading to economic losses. But in July and August 2019, the total monthly rainfall was 1711 and 1312 mm respectively, supported by cloudy weather and high humidity above 93% which led to the sudden outbreak of defoliation followed by dieback of the new vegetative flush of the plants, throughout the region. To combat this unexpected natural calamity thorough investigations were conducted. Extensive surveys conducted during the season revealed that in the primary phase of the disease, new as well as old cashew plants suffered from severe defoliation. On the establishment of the pathogen, the infection progressed to the newly emerged main and lateral branches which consequently lost vigor, turned brown, and then black. The colonization of pathogens within the tissues of the host leads to the formation of small white encrustations on the upper surface of the dead twigs. The pathogen was isolated and identified as *Cylindrocladium* spp.

**Comment [U1]:** If free from destructive diseases the its shouldn't lead to economic losses. Check the sentence

Keywords: Cashew, Konkan, Defoliation, Die-back, *Cylindrocladium* spp.

### Introduction:

*Anacardium occidentale* L. belonging to the family Anacardiaceae is a native to Brazil and was introduced to India five centuries ago (Shanthi and Vittal, 2012). In India cashews are being grown on an area of 10.27 lakh hectares with a total production of 7.25 lakh MT of raw nuts and productivity of 706 kg/ha. India is the largest producer of raw cashew nuts contributing 20 percent of total global production. In India, Maharashtra ranks first in the production and productivity of cashews with an average annual yield of 1262 Kg/ha (Anonymous, 2019).

Cashew is not only a tasty and nutritious food, but it is also a hardy and profitable crop because of its low crop management requirements. Since cashew is a tropical crop, it can withstand higher

**Comment [U2]:** What is the author(s) recommendation?

temperatures, but it is extremely vulnerable to frost. The ideal temperature range for good cultivation is between 20 and 30°C. Cashew thrives in climates with 1000 to 2000 mm of annual precipitation. The coincidence of flowering with high rainfall or excess humidity leads to the incidence of pests and diseases (Bhoomika and Sudha Rani, 2018).

**Comment [U3]:** Authors should write more on the literature review of **Defoliation and die-back and its effect on cashew.**

Incessant prolonged rain showers coupled with more than 90 percent humidity during the monsoon season of 2019, resulted in the occurrence and quick spread of defoliation followed by die-back in all the cashew pockets along the length and breadth of the Konkan region. This situation alarmed an urgent study of the malady to solace the cashew growers with an appropriate and affordable solution to the problem.

**Comment [U4]:** Has this been confirmed in literature? If yes, then source of information should be provided

#### **Material and methods:**

The roving survey was conducted to assess the extent of disease severity in cashew-growing pockets in the region. The disease samples including diseased leaves, dried twigs and branches were collected from the field and were examined to study the symptoms and for isolation and further studies. These samples were then washed with tap water to remove the extraneous material. Temporary mounts were prepared in lactophenol cotton blue as described by Leck *et al.*, 2002 and examined under a microscope to check the presence of microorganisms if any. The pathogen was isolated by following the standard tissue isolation technique. The plates were incubated at room temperature at  $27 \pm 2^\circ \text{C}$  till the fungal mycelium fully covered the surface of the medium. Periodic transfer maintained the fungal culture. Pathogenicity of *Cylindrocladium*spp was proved on one-year-old cashew seedling. Artificially inoculated cashew seedlings exhibited typical symptoms within 10-12 days of inoculation. After the development of symptoms on artificially inoculated leaves, they were compared with naturally developed symptoms under field conditions. Samples taken from artificially inoculated leaves were used for re-isolation of the organism on PDA. After 10 days of incubation, the morphological characteristics of the re-isolated culture were compared with the pure culture of the test organism isolated from naturally infected plants showing defoliation and dieback. The morphology of the re-isolated organism was compared with the information available in the reviewed literature, as well as on standard websites for fungal identification and the organism was identified, up to genus level. Morphological characters of the isolated organism were studied by microscopic examination under 400 X magnification using a digital camera with a measuring

**Comment [U5]:** Which field? Is it the institution farm field or some farmers' field?

device (Catalyst Biotech make –Catcam) attached to Labovision binocular compound microscope with an image analyzer.

### Results and Discussion:

Microscopic examinations of the temporary mounts revealed the presence of hyaline and septate mycelium. Conidia were hyaline, straight, and cylindrical; having 2 to 5 septa this indicated the association of a fungus with the disease. Morphological characteristics of the isolated fungus were compared with the description in reviewed literature as well as the information available on standard websites for fungal identification such as [www.indexfungorum.com](http://www.indexfungorum.com) and [www.mycobank.org](http://www.mycobank.org). Based on this comparison the pathogen was identified as *Cylindrocladium* spp.

Symptomatology was established with periodic observations; Initially, irregularly shaped, minute, dark brown spots appeared which later expanded, coalesced turned brown and formed dark brown blighted patches covering the maximum area of the upper leaf surface while on the dorsal surface of some infected leaves; scanty to dense white mycelial growth was observed. Such infected leaves were shed off within a span of a week or so. This resulted in severe defoliation in some cashew orchards leading to die-back of new vegetative flush of the infected trees. On the establishment of the pathogen, the infection progressed to the newly emerged main and lateral branches which consequently lost vigor, turned brown and then black. The colonization of pathogens within the tissues of the host results in the formation of white encrustations resembling cankerous growth on dried, dead branches. These initially white encrustations later turned pinkish brown probably due to sporulation of the fungus.

**Comment [U6]:** This result show be compared with one obtained in literature review.

All the fungal colonies obtained in repeated isolations of the infected samples were similar in appearance. Initially, white colonies turned pinkish. The Pathogenicity of the test fungus was confirmed on a one-year-old seedling of cashew. Artificially inoculated cashew seedlings exhibited typical symptoms within 10-12 days of inoculation. Symptoms developed as scattered, brown, circular to irregular spots, which later coalesced to cover the maximum surface of the leaf lamina. The inoculated leaves dried, cringed and hung on the stem. The defoliation of the infected leaves as observed in the field was not observed. But die-back symptoms were noticed, overall, the symptoms of artificially inoculated and naturally infected plants were

**Comment [U7]:** See the comment above

similar. A comparison of the culture of the pathogen obtained in isolation and re-isolation revealed the same fungus.

**Comment [U8]:** The figures were not mentioned in the write up.



Fig 1. Field symptoms

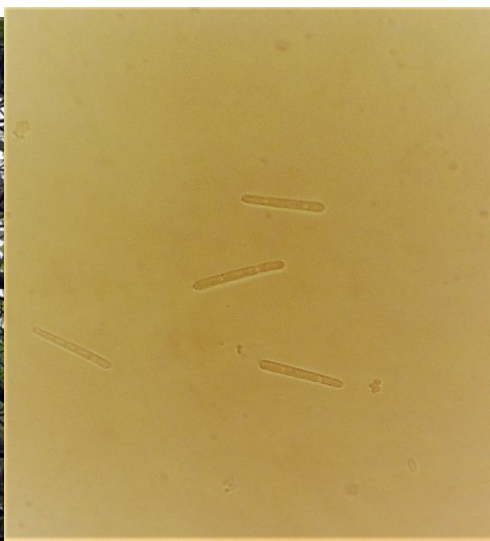


Fig 2. Conidia of *Cyindrocladium* spp

Cashew (*Anacardium occidentale*) is a million-dollar crop, and it is the second most important cash crop of the Konkan region of Maharashtra after mango. After implementation of Employment Guarantee Scheme in Maharashtra in 1990, many small and marginal farmers in the hilly terrain of this region switched over to this hardy and highly remunerative crop owing to its minimal crop management requirements. As a result, the barren and fallow land has been brought under extensive cashew cultivation.

Till recently, the cashew plantations in Konkan region were free from destructive diseases leading to economic losses. But in July and August 2019, the total monthly rainfall was 1711 and 1312 mm respectively, supported by cloudy weather and high humidity above 93 % lead to the sudden outbreak of a new disease throughout the region. Extended rainfall and humid climatic conditions prevailed during monsoon of 2019 favoured establishment of the pathogen in cashew plants and its swift spread in cashew plantations. The infection progressed to the newly emerged main and lateral branches which consequently lost vigor, turned brown and then black. The colonization of pathogens within the tissues of the host leads to the formation of small white

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encrustations on the upper surface of the dried dead twigs. Infection on leaves and stems culminated in defoliation and die-back.

The pathogen *Cylindrocladium* spp was reported to cause disease on different plant species by many workers. Suseela and Dhanesh (2008) reported that *Cylindrocladiumquinquiseptatum* causes brown spot pathogen of vanilla beans. *Cylindrocladium* spp was reported on leaves as well as on twigs of eucalyptus by Mohanty *et al.*, (2012). As per the findings of Arya *et al.* (2012), *Cylindrocladiumquinqueseptatum* causes leaf blight of eucalyptus. Jayaratne *et al.* (2012), isolated *Cylindrocladiumquinqueseptatum* from clove and rubber leaves. Khare *et al.* (2014) conducted cross-inoculation tests and reported that *Cylindrocladiumquinqueseptatum* is a leaf blight pathogen of clove and cashew is one of the hosts of this pathogen. The findings of the present investigation also confirmed that a member of the genus *Cylindrocladium* is responsible for the defoliation and die-back of cashews.

**Comment [U12]:** These should be under introduction

In the present investigation, the pathogenicity of *Cylindrocladium* spp isolated from infected cashew plants was proved by spraying spore cum mycelial suspension of the pathogen. Sharma and Mohanan (1991); Chen *et al.* (2011); Khare *et al.* (2014) and Lopes *et al.* (2018) also reported that spraying the spore suspension on artificially injured leaves is useful to prove the pathogenicity of *Cylindrocladium* spp. The results of the present study are in concurrence with the findings of earlier workers.

While experimenting on leaf blight eucalyptus caused by *Cylindrocladium*, Sharma, and Mohanan (1991) found that the infection of this pathogen results in extensive to complete premature defoliation in eucalyptus accompanied by die-back of tender shoots during the peak period of monsoon (July/August). The disease initiates in the form of minute greyish-black water-soaked lesions on the leaves of any age. Later, the lesions coalesce to form expanded necrotic areas, which dry off, and turn brown, giving a typical blighted appearance. In highly humid conditions, these spots cover the entire leaf lamina and consequently, there is premature defoliation of infected branches. The symptoms of *Cylindrocladium* spp infection on *Pistacia lentiscus* included leaf spots, stem lesions, and severe defoliation of young 10–12 months old seedlings (Vitale and Polizzi, 2008). Mohan and Manokaran (2013), who studied *Cylindrocladium* leaf blight of eucalyptus also noticed that under favorable conditions of high

**Comment [U13]:** Is this result similar or differ from the result obtained in this research?

**Comment [U14]:** What effect those this have on your result?

humidity and frequent rainfall, necrotic lesions cover the entire area of the leaf and young shoot tips resulting in leaf and shoot blight.

In the survey conducted during the present investigation, it was observed that the infected cashew leaves initially exhibit brown-colored spots which later coalesce to cover the entire leaf lamina, and such leaves are shed off when high humidity accompanied by intermittent torrential rain showers prevail in cashew plantations, particularly in the months of July and August. Hence the findings of the present study conform with those of earlier workers who described the symptomatology of the disease incited by this pathogen. Further, it was confirmed that the high humidity favors the multiplication and spread of *Cylindrocladium* spp. One peculiar finding of the present study is the development of white encrustations comprising masses of fungal mycelium on dried dead branches of cashew plants. These initially white encrustations turn pinkish when the mycelium enters the sporulation phase.

With respect to morphological characters, the earliest description of the morphology of *C. quinqueseptatum* on clove was described by Boedijn and Reitsma (1950). Their description states that the mycelium of this fungus formed conidiophores which measured 5-8  $\mu$  in length, broad near the base, and dichotomously branched near the apex. On such conidiophores, primary, and secondary branches are formed but tertiary branches may or may not be formed. Then, the phialides are formed on which cylindrical conidia, with rounded poles and mostly five cross walls are formed.

As per the description given by Crous and Wingfield, (1994), the conidiophores are septate, hyaline, measuring 150- 380  $\mu$ m in length, and terminating into a clavate vesicle, 3-6  $\mu$ m in diameter. They branch into primary, secondary and tertiary, non-septate branches forming cylindrical to allantoid or slightly doliform (barrel-shaped) hyaline, non-septate phialides measuring 13-26 x 4-5  $\mu$ m. Conidia are cylindrical, hyaline, 1-6 septate, rounded at both ends and measure 61-101 x 5-7  $\mu$ m.

Mohanty *et al.*, (2012) also state that the conidiophores are hyaline, non-septate, or single septate arising from a lateral hypha that terminates into a clavate-shaped vesicle bearing hyaline 2.5-3 mm wide phialides. The conidia of *Cylindrocladiumquinqueseptatum* are straight, hyaline, 5 to 6 septate, measuring 560-1040  $\times$  45-70  $\mu$ m (*l X b*).

In the present study, it was revealed from the microscopic observations that the mycelium of *Cylindrocladium* spp was hyaline septate and branched. Conidiophores were branched with non-septate or single septate primary branches and non-septate secondary and tertiary branches. The phialides were hyaline. Conidia were hyaline, straight, and cylindrical and usually five-septate, but some conidia were 2-septate.

### Conclusion

Defoliation and die-back is an emerging serious disease of cashews in the Konkan region, and need to be studied in detail to understand host-pathogen interaction epidemiology and host resistance.

Comment [U15]: This should be re-written out rightly.

### Data availability:

The data supporting the findings of this study can be availed from the corresponding author upon reasonable request. Access to the data is subject to approval by the institutional review board and compliance with data use agreements.

### An ethical approval:

This article does not contain any studies with human participants or animals performed by any of the authors.

### Disclaimer:

The contents, opinions and views expressed in the research article published in Journal are the views of the authors and do not necessarily reflect the views of the organizations they belong to.

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