First report of *Armillaria tabescens* (Scop.) causing root rot on *Dalbergia sissoo* Roxb. in India

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Dalbergia sissoo Roxb. (shisham) is an ecologically, economically and socially accepted important indigenous timber species in the Indian sub- continent. It provides fuel, fodder, fertilizer and timber to the rural community. It is widely planted in forest lands, farm lands and canal/road sides in India, Nepal, Pakistan and Bangladesh. Since 1990s, the population of valuable shisham is dwindling down in unprecedented manner due to die-back disease. Its mortality varied from 10to 22% in Bihar, Haryana, Punjab, Uttar Pradesh, West Bengal and Assam (Chaturvedi *et al.*, 2002).

A survey by the Forest Research Institute (FRI), Dehradun have found that the mortality of shisham has caused a loss of more than Rs 800 -1,000 crore in the Indian sub-continent. Nearly 8 to 10 lakh trees have wilted so far causing a huge loss in India, Bangladesh and Nepal (Business Standard, 2008). Despite the accumulation of knowledge, the cause of die-back may be multi-faceted and the primary causes are yet to be confirmed. Possible pathogens associated with the die-back include a wide range of fungi, but particularly the *Fusarium* and *Ganoderma* spp. These were strongly implicated, but the pathogenicity tests have not been completed to confirm it (Appanah et al. 2009; Singh et al. 2012).

The present study was carried out in 32-year old shisham stand at Forest Research Centre Campus, Mandar (N 23^0 27’ 41.3” and E 085^0 05’ 57.0”), Ranchi (Jharkhand), India during period from 7th July to end of Aug, 2012 to identify the casual organism for shisham mortality. A systematic survey was made in a shisham stand to assess the causes of mortality. The shisham trees were planted in 1980-81 with spacing 3 x 3 m. Soil type is laterite in nature. Total area of the stand is about 1ha (2.5acre). The elevation of the study site is 702m MSL. The climate is humid to sub humid tropical type. The mean annual rainfall is 1017mm. Annual temperature ranges from maximum 42 to 20^{0}C during summer and 25 to 4^{0}C during winter season.

The identity of pathogen was confirmed after investigation and systematic study. In the stand, the author found three distinct circular disease centres, which had about 10-12m radius. Trees were started dying one by one from the disease centre and moving on all directions, if trees have root contact with each other. All trees were found dead in disease centre (Fig 1A). Overall 60% of the standing trees were found dead in the stand. Further, severity of disease was more pronounced in the rainy seasons. Hundreds of thick populated honey colored mushroom fruiting bodies was found in clusters at base and along lateral dead roots of dead trees and partially dead trees. However, no fruiting body was found in healthy trees even if it is standing close to dead tree. Number of fruiting bodies per cluster varies from 5 to 9. However, five per cluster was most common feature (Fig. 2). A recently collapsed shisham tree, 95cm girth at breast height (GBH) and 18.5 m height with heavy fruiting with thin crown and had thick population of fungi fruiting bodies at base (looking healthy the previous year), was chosen to assess the extent infection and damage. The root system of the collapsed tree was excavated by hand to 0.5m depth from the base of the tree and 30 x 30 cm length of bark was also removed at different height positions viz., collar, GBH, mid – height and dead branch (30cm girth) by hand with help of ladder to look for presence of mycelia fans, decay, and rhizomorphs distribution.

Macro-morphological characteristics and keys of the Armillaria root rot and external symptoms of infected tree reported earlier were used for identification of casual agent of die-back in shisham in the present study (Watling *et al*. 1991; Tsopelas and Tjamos, 1997; Cha *et al*. 2009). The key morphological features of mushroom for identification were honey/yellow –brown cap, small scales/fibrils on cap, gills whitish-attached to stem but notched, presence or absence of ring on stem, mushrooms in clusters, white spore print and presence of mycelial fans (like dried soft white peelabe paint) between bark and wood (Fig 2) and distribution of rhizomorphs. The external symptoms of the infected tree used are die-back of twigs and branches, stunted leaves, heavy fruiting, crown thinning, chlorosis and pre-mature fall, burnt appearance of immature fruits (Fig 1) and gummosis on stem (Fig 2C).

As the above-said morphological features and typical external symptoms of *Armillaria* fungi were observed and documented in all dead trees during survey. *Armillaria tabescens* is the only species under the genus *Armillaria* that do not have rings on stem (Watling *et al*. 1991; Tsopelas and Tjamos, 1997; Cha *et al*. 2009). This typical morphological feature was recorded in the present study. Based on this fact, the author has easily identified the casual agent for shisham root rot as *A. tabescens* (Fig 3). *Armillaria tabescens* is distributed worldwide (Ota *et al*. 1988; Kim *et al*. 2010) with wide host range (Antonin *et al*. 2006). *Armillaria tabescens*is usually found in oak dominated forests (Keca *et al*. 2009) and observed mostly on butts and root systems of dead or dying trees and on stumps.

The rhizosphere soil around root zone of the dead tree was collected along the fruiting bodies and placed in the root system of healthy trees. It was found that all inoculated trees shown typical symptoms and out of 5 trees, 3trees were dead within 2-months.It proved that the *Armillaria* is soil borne and spread through root–root contact mode. There was no observation of fruiting bodies and rhizomorphs on apparently healthy trees. However, the presence of fine black parallel lines of rhizomorphs beneath living bark and cambium confirmed its colonization on all living weakened neighbouring trees without apparent symptoms.

The higher number of fruiting bodies (basiodiomes) and density of rhizomorphs of the infected trees showed higher level colonization and vice-versa, i.e. dead and dying trees had higher number of basiodiomes and rhizomorph line on stem and roots than that of colonized living trees(Fig.2 & 5).As the large scale shisham mortality was reported in Bihar (adjusting State to Jharkhand), the author personally was made a survey and found and recorded similar mushrooms and external tree symptoms in Vaishali and Patna. In contrast to reports of Antonin and others, no rhizomorphs of *A. tabescens* were found in the soil in the present study by the author. The observations of Tsykunand others (2010) are in conformity with present studies. The live fruiting bodies were preserved in 2% formalin and spirit for future studies. Although, there are previous records of *Armilaria* root rot on many tree species in other parts of the world, this is the first report of *A.tabescens* as the cause of root rot in *D.sissoo* in India. Although the pathogen was identified based on morphological features and tree symptoms, it need to be confirmed and authenticated with molecular studies and pathogenicity tests.

CONCLUSION

Genetic diversity of shisham is dwindling down rapidly due to large scale mortality both in farm lands and forests and its exclusion from choice of species afforestation programme by both the farmers and forest departments. *Armillaria tabescens* was found to be the causative organism of root rot in shisham that leads to mortality and is the first report recorded in India. A holistic detailed investigative study is needed to understand ecology, host range pathogenicity of this fungus and its related species and to develop control measures to conserve this valuable species.

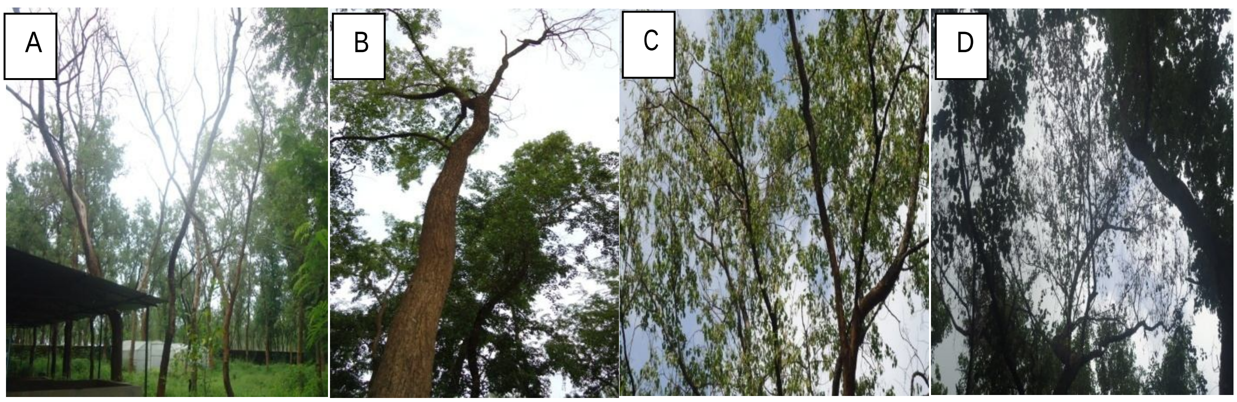

Fig. 1: Disease Centre (A), partially dead tree (B) and heavy fruitingand crown thinning (C &D)

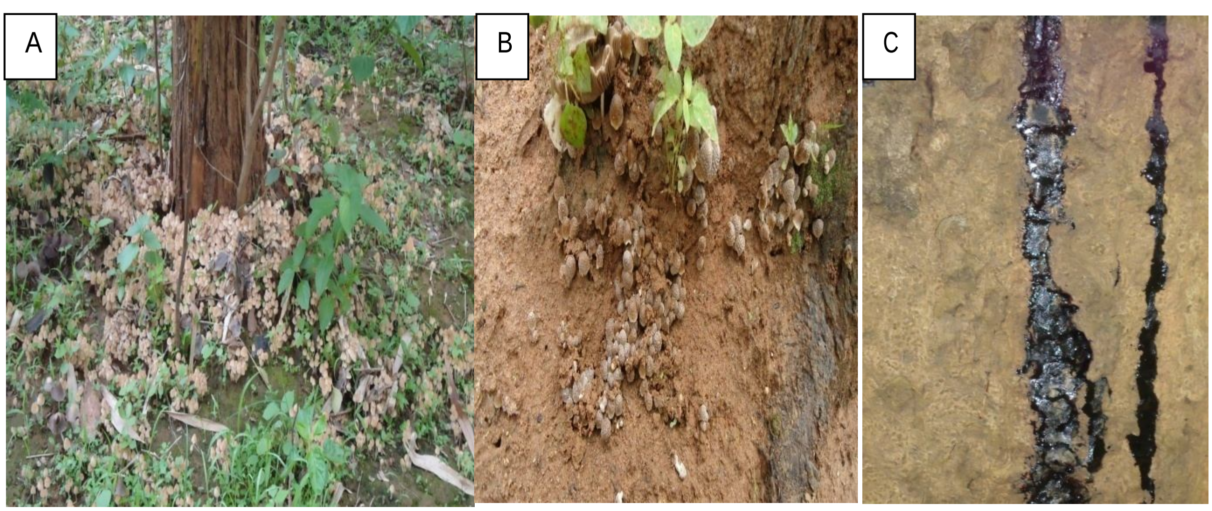

Fig.2:Mushrooms population at base (A & B) andgummosis on stem of infected tree (C)

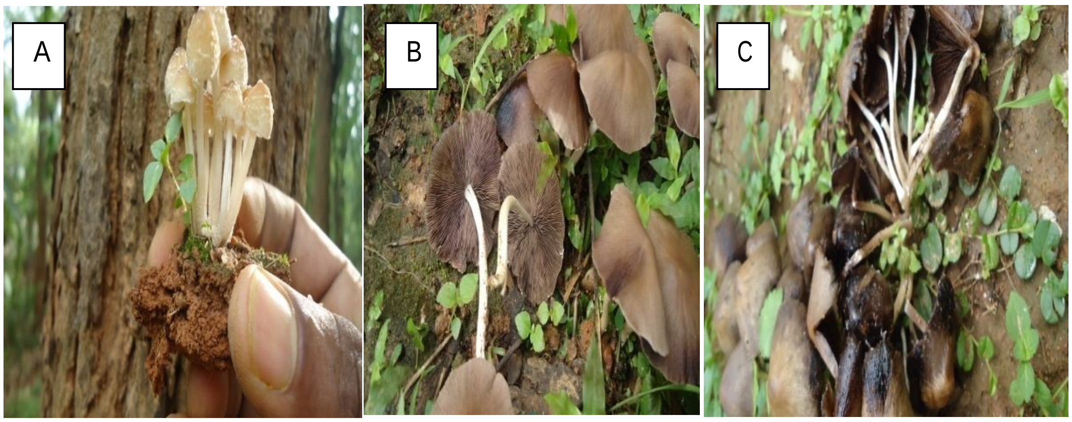

Fig. 3: One-day with fibrils (A), 2-day old (B) and 3-day (C) ring-less mushrooms

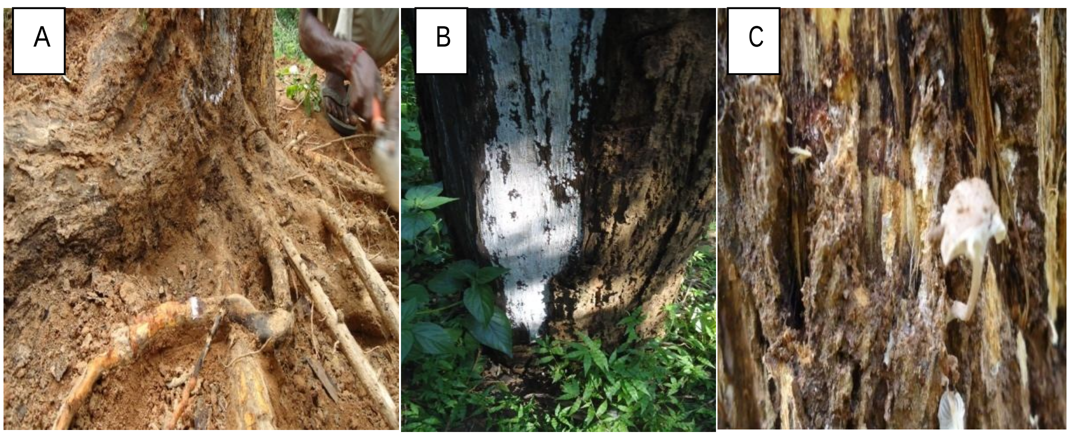

Fig.4. Infected root system (A), mycelial fansunder bark of root (B & C)

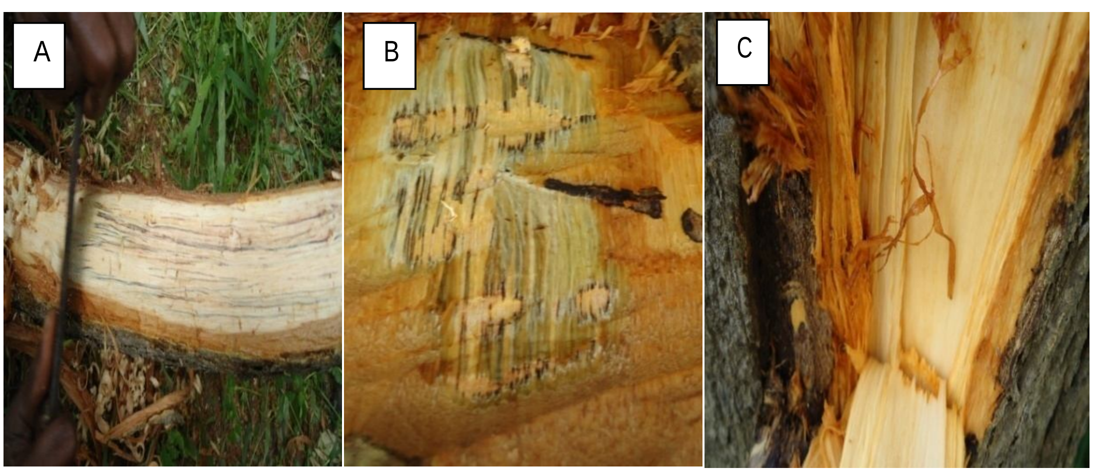

Fig.5:Rhizomorphs on infected branch wood (A), Stem wood (B) and healthy tree stem (C)

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