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CAUSE OF EUCALYPTUS CITRIODORA DIEBACK IN PUNJAB, PAKISTAN

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A B S T R A C T

The present study was carried out to explore the causal organism of dieback of *Eucalyptus citriodora* Hook in different areas of Punjab, Pakistan. Diseased samples of *E. citriodora* suffering from dieback were collected during January to March 2010. Purple to reddish spots were observed on leaf of this tree. Pathogen was isolated on malt extract agar medium. Isolated fungus was identified as *Alternaria alternata* (Fr.) Keissl. The isolated fungus was inoculated on *E. citriodora* leaves. The same fungus was re-isolated from the artificially inoculated diseased leaves that confirms *A. alternata* as causal agent of dieback in *E. citriodora* in Pakistan.

Keywords: *Alternaria alternata,* dieback, *Eucalyptus citriodora,* Pakistan.

INTRODUCTION

Eucalyptus (family Myrtaceae) is among the world's most widely planted genera that contains about 900 species and sub species (Zhao *et al.,* 2010). This group of aromatic and ever green plants is native to Australia with some species growing naturally in Philippines, Papua New Guinea and East Timor (Duke 1984; Turnbull, 2000). These important tree species are used for control of wind and water erosion, production of timber, fuel and paper pulp, as well as a source of essential oils (Brooker and Kleinig, 2006; Martinez *et al.,* 2015), and for salinity phytoremediation (Doronila and Forster, 2015). *Eucalyptus* consists of tree species with fragrant foliage which are rich in oil glands and thus serve as tremendous source of commercial *Eucalyptus* oil (Batish *et al.*, 2008; Ali *et al.,* 2014). *Eucalyptus* oil acts as pesticidal agent against fungi, bacteria and insects due to the presence of terpenoids predominantly monoterpenes and sesquiterpenes, and a variety of aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketone in it (Brooker and Kleinig, 2006; Batish, 2008; Jinbiao *et al.,* 2010). *Eucalyptus* oil can also be used as natural insect repellent due to its insecticidal properties and provide protection against mosquitoes and other harmful arthropods (Toloza *et al.,* 2010; Maciel *et al.,* 2010).

Eucalypts species are dying these days due to several diseases. Severe loses to these plants are mainly occurred due to bacterial and fungal diseases (Van Heerden *et al.,* 2005; Dick *et al.,* 2006; Brady *et al.,* 2009). In Pakistan, *Eucalyptus* spp. especially *E. citriodora* and *E. camuldulensis* are suffering from dieback (Javaid *et al.,* 2004). Dieback usually refers to prolonged malfunction in stands of trees due to the persistent action of damaging factors and causes deaths of trees (Rice *et al.,* 2004). There could be number of factors of dieback, these may include immediate impacts of acute stress, unnatural chemical imbalances in the atmosphere, soil and water, and individual biotic agents like fungi, bacteria, virus and insects. Dieback-affected trees typically have poor crowns, with sparse foliage and a large proportion of dead twigs and branches (Javaid *et al.,* 2004). Among the various *Eucalyptus* species being cultivated in Pakistan, *E. citriodora* is the most common and is highly valued for its citronellal-rich essential oil (Ali *et al.,* 2014). The present study was, therefore, designed to diagnose which pathogen is primarily responsible for dieback of *E. citriodora* in different areas of Punjab, Pakistan.

MATERIAL AND METHODS

Collection of diseased plant samples: Surveys of different areas of Punjab viz. Lahore, Sialkot and Rawalpindi were carried out in 2010 to collect samples

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from *E. citriodora* suffering from dieback. Surveys were carried out on plants growing along the roads and canal banks, and in private plantations and parks. Visual symptoms of the disease on dying back *E. citriodora* trees were recorded. Infected leaf and stem samples were collected for pathogenic studies, and carried to the laboratory in sterilized polythene bags.

Isolation of the pathogen(s): For isolation of pathogenic bacteria, leaves and stems of diseased plants were cut in small pieces and surface sterilized with 0.01% sodium hypochlorite for 1 min and rinsed several times with distilled, autoclaved water. The surface sterilized tissues were immersed in a sterile tube and crushed with clean glass rod. The resulted mixture was allowed to stand for 10 min to allow the bacteria to diffuse out of tissue into the water. The supernatant was transferred to 1.5 mL eppendorfs. These eppendorfs were subjected to centrifugation at 12000 rpm for 3 min to pellet the bacteria. After centrifugation, supernatant was discarded. The resulted pellet was directly streaked on to two different mediums, LBA and glucose yeast extract carbonate agar medium (GYCA) separately under aseptic conditions. The plates were incubated at 30 ºC for three days.

For isolation of pathogenic fungi, the surface sterilized portions of the infected stems and leaves were placed on malt extract agar medium in 9-cm diameter Petri plates. Plates were incubated at 25 ºC for 7 days. The pathogen(s) were identified morphologically on the basis of size, shape and colour of colony and conidia following Domsch *et al.* (1980).

Assay for Pathogenicity: Inoculum of the isolated fungus was produced on malt extract agar medium amended with 250 mg L^{-1} of chloramphenicol to avoid bacterial contamination. Conidia were collected from a 14-days old culture by adding sterilized water and scraping the agar surface with a rubber spatula. Conidial suspension was filtered through a single layer of sterile cheesecloth and final conidial suspension was adjusted to 10^6 conidia mL^{-1} with the aid of hemacytometer. Pots of 20-cm diameter and 15 cm deep were filled with sandy loam soil collected from a cultivated field of Punjab University, Lahore. *E. citriodora* plants were transferred to these pots. Pots were kept in shade for 7 days for the establishment of plants and were irrigated with tap water, when required. After the establishment of plants, leaves were inoculated with spore suspension of 10^6 conidia mL^{-1} and incubated under normal environmental conditions. Plants were covered with plastic bags to sustain high humidity for 24 hours. Thereafter, bags were removed and plants were kept under observation for 30 days. Control plants were sprayed with sterilized water. The pathogenicity tests were repeated three times. The first lesions appeared after a period of 15 days. The pathogen was consistently re-isolated from the lesions.

RESULTS AND DISCUSSION

Disease symptoms: Purple to reddish spots were present at the early stage of the infection which frequently coalescing to form larger blotches across the leaf surface, first at the top and margins and then moved towards the centre of the leaf. Defoliation caused the dieback and eventually the death of the tree takes place (Figure 1 and 2).

Figure 1. A dying back *Eucalyptus citriodora* tree.

Figure 2. Spots on leaves of *E. citriodora.*

Identification of the pathogen: None of the bacterial species was isolated on LBA and GYCA from infected leaf and stem samples. However, fungal colonies were appeared on malt extract agar medium from infected leaves. Colony surface was greenish black with a light border, while the reverse side was brown to black in colour. Microscopically the fungal mycelium was septate, brown hyphae bearing simple large brown conidia with transverse septations were identified using lactophenol preparations. Mature conidia were short beaked and wide ellipsoid to ovoid in shape with short chains. The ends of the conidia near conidiophore were round while it tapered towards the apex. The fungus was identified as *Alternaria alternata* (Figure 3).

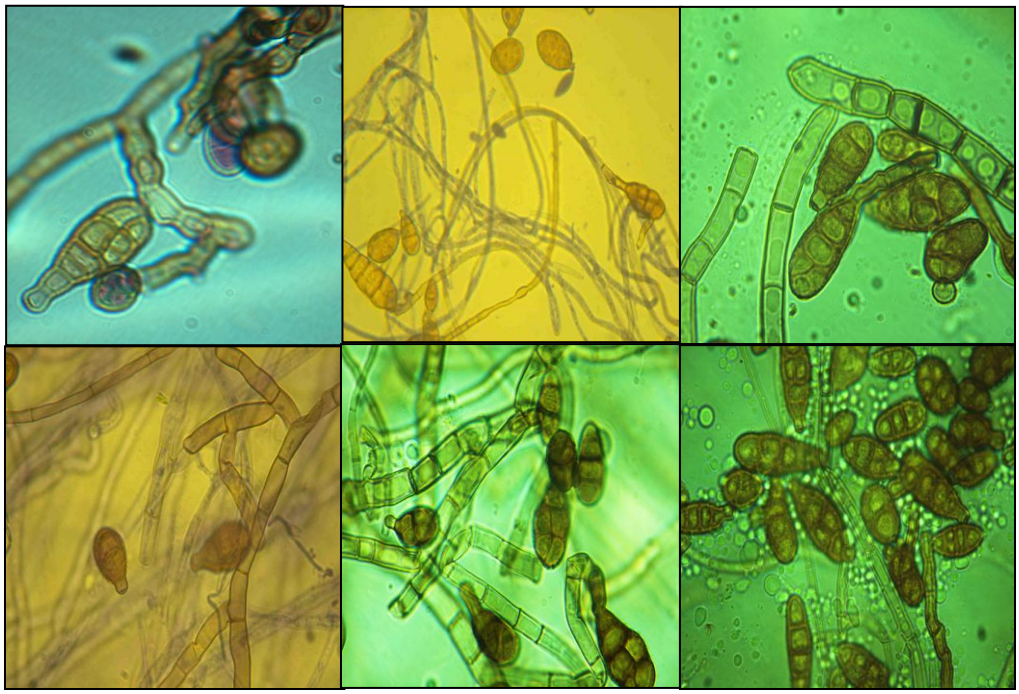

Figure 3. Conidia of *Alternaria alternata* isolated from different samples of *E. citriodora.*

Koch's postulates applied to test the pathogenicity of *A. alternata* gave positive results. Disease symptoms were gradually appeared on leaves. The pathogen was re-isolated from infected plant tissues. Earlier there are many records of *A. alternata* on *Eucalyptus* in the world. Attrackchi and Tarabeih (1986) discovered that *A. alternata* causing leaf spots on *Eucalyptus* in Iraq. Similarly *A. alternata* and *Alternaria tenuissima* cause leaf spot in Tarai areas of India on *Eucalyptus tereticornis* (Bakshi, 1972). *A. alternata* and *A. tenuissima* were isolated from leaf surface of *Eucalyptus panciflora*, *E. stelullata* and *E. regnans* in Australia (Lamb and Brown, 1970). Abd-Allah and Salih (2008) isolated *A. alternata* from the infected leaves of *Eucalyptus globulus* from Saudi Arabia. The present study concludes that *A. alternata* is the cause of dieback in *E. citriodora* in Pakistan.

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