

Isolation experiments fungi from bark beetles

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Abstract: Bark beetles (Coleoptera: Curculionidae: Scolytinae) are among the most destructive insects in boreal and temperate regions especially conifer forest ecosystems in northern hemisphere and may cause huge economic losses. Bark beetles are completed the majority of their lives under the bark or phloem of coniferous and deciduous trees. It is known that the bark beetles are vector to some fungal species during the transition from tree to tree for reproduction. These fungal species are ecologically different. Some of them are nutrient resource for bark beetles, while others are important pathogenic species for woody plants. The most known plant pathogenic fungi that associated with bark beetles are *Ophiostoma* species. The aim of our research is to isolate the fungus associated with bark beetles. In the study, bark beetles were obtained from the black pines in the forests of Balıkesir Regional Directorate in 2014. Our research was conducted on 4 different black pine (*Pinus nigra* J. F. Arnold.) stands where bark beetle damage was determined. Wood traps were established to the experiment area at the end of February. Wood traps had been checked regularly from March. Fungal isolates were grown from *Ips sexdentatus*, *Orthotomicus erosus*, *Hylurgus ligniperda* and *Hylurgus Micklitzi*. The bark beetles were surface sterilized with 0.5% sodium hypochlorite. While some of them were placed individually into petri dishes containing about 25 ml 1% MEA (Malt Extract Agar), the others were transferred separately into petri dishes containing 1% MEA added 100 µg/ml streptomycin to inhibit bacterial growth and 500 µg/ml cycloheximide for inhibit some fungal growth. Petri dishes were incubated at 20 °C and checked daily for two months. Out growing fungal mycelia were sub-cultured in new petri dishes with fresh 2% MEA. The fungal cultures were divided into the groups according to their morphology to be subjected to DNA sequence analysis for species identification. Fungal species growing on first type medium are known as contaminant species; *Aspergillus*, *Penicillium*, *Rhizopus* and also *Trichoderma* spp. In addition, in some petri dishes, different colonies immersed into medium were observed but pure cultures were not obtained. Bacterial contamination occurred in some of those petri dishes. On the other medium 8 different types of colony morphology were determined. Each group representing the fungus was extracted using the NucleoSpin Plant II - Macherey-Nagel Mini Kit. Genomic DNA samples of each isolate were identified by amplifying elongation factor primers. The findings will shed light on the fungus-insect relationship that can cause deaths on black pine in Balıkesir region.

Keywords: Bark beetles, Fungi, black pine, Balıkesir

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