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Growth patterns of *Aspergillus sp.* in stored rice during the post-harvest storage and their in-vivo growth control by cinnamon oil-chitosan microcapsules

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Rice (Oryzae sativa L.) is the most important staple food crop in Sri Lanka. Inadequate storage conditions for rice can result in fungal infestation, leading to the presence of micotoxin that pose significant health risks to humans. A common storage fungus, Aspergillus sp. is responsible for producing Aflatoxins in stored grains. The use of cinnamon leaf oil (CNO) as an antifungal agent has garnered attention due to its low toxicity to mammals, high effectiveness, and wide availability. However, the direct application of CNO presents challenges such as volatilization, degradation of active compounds, and imparting unpleasant taste and odor to food products. To overcome these limitations and achieve controlled release, microencapsulation is an effective technique. Understanding and controlling the growth of aflatoxigenic fungi, specifically Aspergillus sp. on stored rice is of paramount importance to ensure food safety. In this study, a comprehensive investigation was conducted to assess the in-vivo growth dynamics of Aspergillus sp. colonies over a six-month period during the post-harvest storage. The rice samples were carefully stored in separate poly bags and rice packets to monitor any variations in fungal colonization. Microcapsules (MCs) containing cinnamon leaf oil were prepared by inotropic gelation of chitosan crosslinking with sodium tripolyphosphate (STPP 1%). Monthly observations of Aspergillus sp. in rice were performed by growing in potato dextrose agar (PDA) media and subsequent spore quantification was carried out using a haemocytometer. The results demonstrated that the spore counts exhibited slight variations throughout the six-month duration. Notably, the highest spore count of 3.02 x 107 mL-1 was observed in the sixth month, suggesting a potential accumulation of viable spores during prolonged storage. To investigate the efficacy of growth control measures, rice grains were inoculated with the spores of Aspergillus sp. and assessed the minimum inhibitory dose (MID) of cinnamon oil loaded chitosan microcapsules (CNO-CS-MCs). Remarkably, the Aspergillus sp. fungus exhibited growth when exposed to 1 -5 mg of cinnamon oil within the microcapsules, indicating their role in facilitating fungal proliferation. However, a noticeable inhibition of fungal growth was observed when the oil dose in MCs was increased to 7.5 mg. And free CNO mixed rice grains showed 100 % growth inhibition of Aspergillus sp. at 2 mg of oil (MID). Upon further incubation in PDA media, fungal growth was detected in the 7.5-12.5 mg CNO range. And treatments with 15 mg and higher doses exhibited no fungal growth after incubation in PDA media. Therefore, the minimum lethal dose (MLD) of CNO-loaded chitosan microcapsules against Aspergillus sp. was determined to be 15 mg. These findings emphasize the promising potential of CNO-CS microcapsules as a mean to control the growth of aflatoxigenic fungi on stored rice.

Keywords: Chitosan, Cinnamon oil, Microcapsules, Stored rice

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