ผลของสารสกัดจากใบชาในการยับยั้งเชื้อราสาเหตุโรคขั้วหวีเน่าของกล้วยหอม
Effect of Tea Extract on the Inhibition of Banana Crown Rot Pathogens

Zaryab Raza[1] กัลยา ศรีพงษ์[1] อภิรดี อุทัยรัตนกิจ[1, 2] ณัฏฐา เลาหกุลจิตต์[1] พนิดา บุญฤทธิ์ธงไชย[1, 2] และ ผ่องเพ็ญ จิตอารีย์รัตน์[1, 2, *]
Raza, Z.[1], Sripong, K[1], Uthairatanakij, U.[1, 2], Laohakuljit, N.[1], Boonyaritthongchai, P.[1, 2] and Jitareerat, P.[1, 2, *]

Abstract

A common postharvest disease of Cavendish banana is crown rot caused by three major fungal pathogens including *Colletotrichum musae*, *Fusarium* sp. and *Lasiodiplodia theobromae*. The objective of this study was to investigate the effect of black tea (BT) extract at 0 (control), 1, 5, 10, 20, 50 mg/ml on mycelium growth and spore germination of these pathogens. The result showed that BT extract could inhibit the mycelium growth of those fungi. The inhibitory degree increased as the increase of BT concentration. The maximum inhibition was 50 mg/ml which their inhibitory percentages on mycelium were 51.18%, 41.42% and 26.44% in *Fusarium* sp., *C. musae*, and *L. theobromea* respectively. In addition, BT extract at 50 mg/ml inhibited the spore germination of *Fusarium* sp. and *C. musae* by 26.4 and 8.32-fold of the control. This result implies that BT extract may be a new approach as the alternative treatment for controlling crown rot disease in banana.

Keywords: banana, crown rot, postharvest disease, spore germination

บทคัดย่อ

โรคหลังการเก็บเกี่ยวของกล้วยหอมที่พบเสมอคือ ขั้วหวีเน่า มีสาเหตุจากเชื้อรา 3 ชนิด ได้แก่ *Colletotrichum musae*, *Fusarium* sp. และ *Lasiodiplodia theobromae* งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของสารสกัดใบชาดำที่ 0 (ชุดควบคุม), 1, 5, 10, 20, 50 mg/ml ต่อการเจริญเส้นใยและการงอกของสปอร์เชื้อราเหล่านี้ พบว่าสารสกัดใบชาดำสามารถยับยั้งการเจริญเส้นใยของราทั้งสามนี้ได้ โดยระดับการยับยั้งเพิ่มขึ้นตามความเข้มข้นของสารสกัด โดยที่ความเข้มข้น 50 mg/ml สามารถยับยั้งเส้นใยได้สูงสุด ซึ่งสามารถยับยั้งเส้นใยของเชื้อ *Fusarium* sp., *C. musae* และ *L. theobromea* ได้ 51.18%, 41.42% และ 26.44% ตามลำดับ ขณะที่ที่ความเข้มข้น 50 mg/ml สามารถยับยั้งการงอกของสปอร์ *Fusarium* sp. และ *C. musae* ลงได้ 26.4 และ 8.32 เท่าของสปอร์ชุดควบคุม งานวิจัยนี้แสดงให้เห็นว่าสารสกัดใบชาดำเป็นวิธีที่สามารถใช้ควบคุมโรคขั้วหวีเน่าของกล้วยได้

คำสำคัญ: กล้วย ขั้วหวีเน่า โรคหลังการเก็บเกี่ยว การงอกของสปอร์

Introduction

Bananas belonging to the family *Musaceae,* popular globally not only for their nutritional value but also for their economic importance. These are grown in over 130 countries across the world in an area of 10.1 million ha producing 121.85 million tones (FAO, 2009; Sidhu and Zafar, 2018). In Thailand, bananas have been increasingly important not only for domestic consumption but also for overseas exports. Crown rot is considered to be the main postharvest disease of export banana (Lassois and de Bellaire, 2014). Predominantly fungal agents of crown

[1] คณะทรัพยากรชีวภาพและเทคโนโลยี มหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรี 49 ซ.เทียนทะเล 25 ถ. บางขุนเทียน-ชายทะเล แขวงท่าข้าม กทม.10150
[1] School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, 49 Thian Thale 25, Bangkhun-thien rd., Thakam, Bangkok, 10150 Thailand
[2] ศูนย์นวัตกรรมเทคโนโลยีหลังการเก็บเกี่ยว กระทรวงการอุดมศึกษา วิทยาศาสตร์ วิจัยและนวัตกรรม กรุงเทพมหานคร 10400
[2] Postharvest Technology Innovation Center, Ministry of Higher Education, Science, Research and Innovation, Bangkok 10400, Thailand

rot disease are *C. musae*, *L. theobromae,* and *Fusarium* spp. (Kamel et al., 2016). Fungicides are often applied but it has an adverse effect on the environment and human health. Plant extract can be an alternative method to control fungal pathogens. Previously, tea polyphenol (TP) extract has been applied to test its inhibitory effects on three species of plant pathogenic fungi, *Bipolaris maydis*, *C. musae*, and *F. oxysporum* (Yang and Zhang, 2019). *In vivo* test, TP exhibited an inhibitory effect against stem-end rot (a postharvest disease caused by *Diplodia natalensis*) in citrus fruit at concentrations of 0.1%, 0.5%, and 1.0%. Further, the inhibitory activity of TP was confirmed again to against gray mold disease of grapefruits caused by *Botrytis cinerea.* Previous study demonstrated that TP extract at 0.1% could inhibit the spore germination of *B. cinerea* significantly, and mycelium growth was significantly inhibit. (Liu et al., 2010). Thus, this preliminary research aimed to study the efficiency of black tea extract on mycelium growth and spore germination of *C. musae*, *L. theobromae,* and *Fusarium* sp. which are the causal agents of banana crown rot.

Materials and methods

Preparation of black tea (BT) extract: BT extract was prepared as the method of Bansode (2016). Ten gram of dry black tea was ground using a pastel and motor, and 200 ml of distilled water was added, then boiled at 60ºC for 1 h. Afterward, the sample was cooled down at room temperature, separation of the liquid and solid form was done by using Whatman no 1 paper that was equipped with a suction pump filtration for three times. Subsequently, tea extract takes from the chamber to the rotary evaporator equipped with the water bath at 60 ºC. BT crude extract was kept at -20°C for this study.

Effect of BT extract on mycelium growth and spore germination of crown rot pathogens: *C. musae*, *L. theobromae,* and *Fusarium* sp. were isolated from the crown of banana using tissue transplanting method. For mycelium growth test, the mycelial disc (∅ 0.5 cm) of 5-7 days-old culture of each fungus were placed at the center of potato dextrose agar (PDA) plate, that PDA was contained with BT extract at different concentrations; 0 (control), 1, 5, 10, 20 and 50 mg/ml. The petri dish was incubated at 26±2ºC) for 3-10 days depending on the type of pathogen. The diameter of mycelium growth on the surface of the PDA was measured every day by the ruler until the observed growth in the control reached the edge of the Petri plates. The percentage of mycelial inhibition was calculated as the following formulation: [(dc-dt) dc x100] (Lim et al., 2002), where dc = mean colony diameter of control samples, and dt = mean colony diameter of treated samples. For spore germination test, 0.1 ml of spore suspension at 10^3 spores/ml of each pathogen was spread on the surface of PDA contained with BT extract at 0 (control), 1, 5, 10, 20 and 50 mg/ml. The PDA plates were incubated at 26±2ºC for 1 day. The numbers of the fungal colony on PDA were counted. Each treatment was consisted of four replications. The data was analyzed by Statistical Analysis System (SAS) software. The mean difference was determined using Duncan's multiple tests at $P<0.05$.

Results and Discussion

The antifungal effect of BT extract on the mycelial growth of crown rot pathogens is shown in Figure 1. The inhibitory effect of BT extract against *Fusarium* sp., *C. musae* and *L. theobromae* at concentration of 50 mg/ml was significantly higher than other treatments. Besides, 50 mg/ml BT extract gave better result in inhibiting the growth of *Fusarium* sp. than *C. musae* and *L. theobromae.* The inhibitory effect of BT extract on spore germination is showed in Figure 2. The spore germination rate of *C. musae* and *Fusarium* sp was significantly lower in the BT treatment than that of control. But at the maximum concentration (50 mg/ml) could not inhibit the spore germination of both *Fusarium* sp. and *C. musae* completely. Similar research reported that BT at 0.5%

and 1.0% significantly inhibited spore germination and mycelium growth of *Diplodia natalensis,* cause of stem-end rot in citrus fruit respectively (Liu et al., 2010). Previous studies also showed that tea polyphenol (TP) (5-10 mg/ml) could inhibit the mycelial growth and spore germination of *B. maydis*, *C. musae,* and *F. oxysporum* (Wang et al., 2008), *Pyricularia oryzae*, which causes rice blast disease (Wang et al., 2011) and *Phytophthora cryptogea*, *Pestalotiopsis apiculatus*, *Colletotrichum Horii*, *Sclerotinia sclerotiorum*, *C. fructicola*, *Rhizoctorzia solani*, *L. theobromae*, and *F. oxysporum* (Zou et al., 2017). This research concluded that phenolic and catechins in tea are the key compounds to against the fungi. TP treatment changed the membrane permeability of fungi which can be indirectly detected by measuring the electrolyte leakage. This would disrupt fungal growth or even cell death (Shadmani et al., 2015). However, the present result shows that 50 mg/ml BT extract showed the highest inhibitory effect on mycelium growth and spore germination of crown rot pathogens. Therefore, BT extract may have the good promising to control crown rot disease of banana.

Summary

Concerning environmental protection, finding 'friendly antifungal agents' from plants is an appropriate approach to achieve plant protection without polluting the environment. Our findings suggest that BT extract is high effective against crown rot fungi of bananas. BT extract at 50 mg/ml significantly inhibited the mycelium growth of *Fusarium* sp., *C. musae* and *L. theobromae and* the spore germination of *C. musae* and *Fusarium* sp. when compared with the control. Thus, BT extract is a possible alternative to chemical treatment. For future, the application of BT extract *in vivo* test should be studied.

Acknowledgements

The authors thank to the KMUTT International Scholarship Program (KISP) for the first author and the United Graduated School of Agricultural Science (UGSAS), Gifu University for supporting research equipment.

References

FAO, 2009, Food and Agriculture Statistical Databases (FAOSTAT), In: http://faostatfao.org

Bansode, P., 2016, Spectrophotometric Determination of Total Phenolic Content of Some Commonly Consumed Teas in Imdia, Bionano Frontier, 7(1): 78-80.

Kamel, M.A.M., Cortesi, P. and Saracchi, M. 2016. Etiological Agents of Crown Rot of Organic Bananas in Dominican Republic, Postharvest Biology and Technology, 120 : 112-120.

Lassois, L. and de Lapeyre and Bellaire, L. de L., 2014, Crown Rot Disease of Bananas. *In* Ed. By Silvia Bautista-Banos. Postharvest Decay: Control Strategies. Amsterdam, Elsevier, pp. 102-130.

Liu, H.M., Guo, J. H., Liu, P., Cheng, Y. J., Wang, B. Q., Long, C. A. and Deng, B. X., 2010, Inhibitory Activity of Tea Polyphenol and *Candida ernobii* Against *Diplodia natalensis* Infections, Journal of Applied Microbiology, 108(3): 1066-1072.

Lim, J.Y., Lim, T.H., and Cha, B.J., 2002, Isolation and Identification of *Colletorichum musae* from Imported Bananas, The Plant Pathology Journal, 18(3): 161–164.

Sidhu, J.S. and Zafar, T.A., 2018, Bioactive Compounds in Banana Fruits and Their Health Benefits, Food Quality and Safety, 2(4): 183–188.

Shadmani, N., Ahmad, S. H., Saari, N., Ding, P., and Tajidin, N.E., 2015, Chilling Injury Incidence and Antioxidant Enzyme Activities of *Carica papaya* L. "Frangi" as Influenced by Postharvest Hot Water Treatment and Storage Temperature, Postharvest Biology and Technology, 99: 114–119.

Wang, J., Qiu Y., Chen, H., Lu H., Lin, X. and Ma, L., 2008, Inhibitive Action of Tea-polyphenol on Some Plant Pathogenic Fungi, Natural Product Research, 20: 690 – 694.

Wang, J. Qiu Y., Fu J., Han J. and Feng W., 2011,. Inhibitive Effect of Tea-polyphenols on *Pyricularia oryzae* and Its Mechanism, Natural Product Research Dev., 23: 918 – 922.

Yang, Y. and Zhang, T., 2019, Antimicrobial Activities of Tea Polyphenol on Phytopathogens: A Review, Molecules, 24(4):816-240

Zou, D., Liao W., Huang H. and Jiang X., 2017, The Inhibition Effect of Tea Polyphenol on 8 Kinds of Plant Pathogenic Fungi. Guangxi Forest Science, 46:412–415.

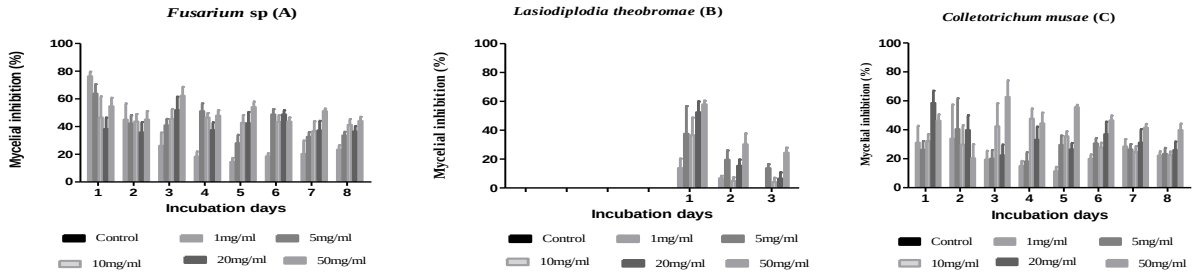

Figure 1 Effect of black tea (BT) extract at 0 (control), 1, 5, 10, 20 and 50 mg/ml on mycelium growth of *Fusarium* sp. (A), *L. theobromae* (B), and *C. musae* (C) during incubated at 26±2°C for 3 or 8 days. Bars represent the standard errors of the means between treatments.

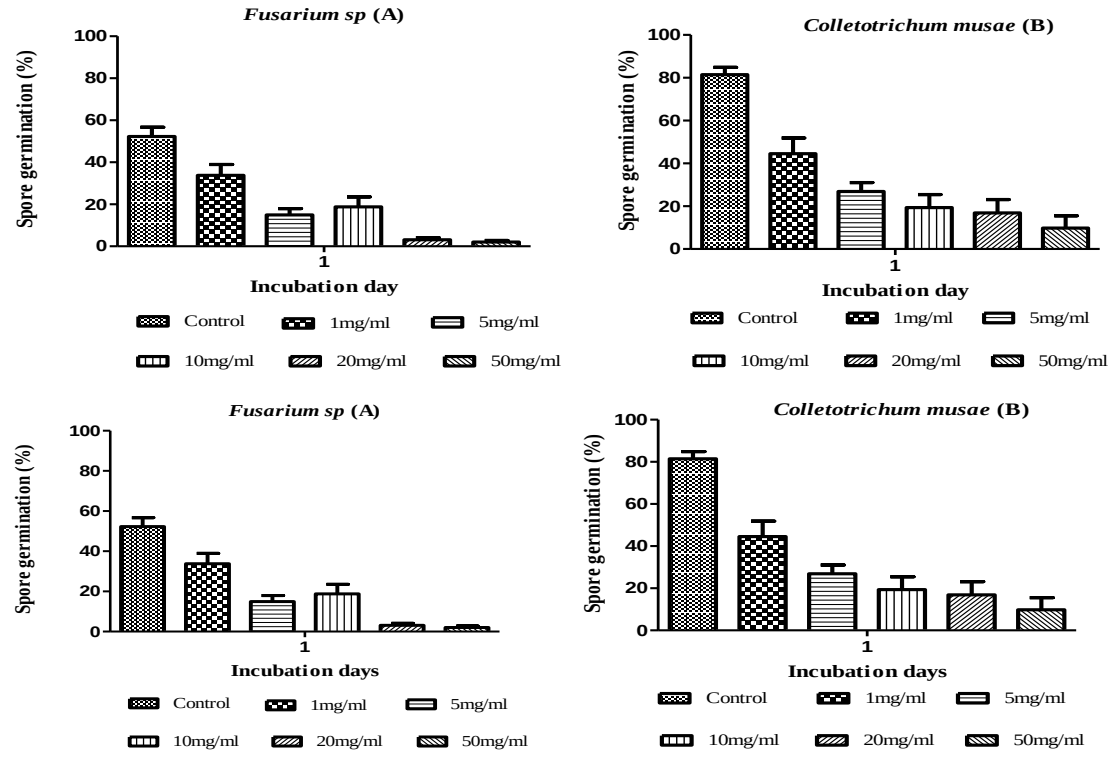

Figure 2 Effect of black tea (BT) extract at 0 (control), 1, 5, 10, 20 and 50 mg/ml on spore germination of *Fusarium* sp. (A) and *C. musae* (B) during incubated at 26±2°C for 1 day. Bars represent the standard errors of the means between treatments.