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ผลของการใช้โอโซนเพื่อควบคุมโรคผลเน่าของขนุนหลังการเก็บเกี่ยว
The Effect of Ozone Treatment on Fruit Rot Disease Control in Harvested Jackfruits

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บทคัดย่อ

การศึกษาผลของโอโซนต่อการควบคุมโรคผลเน่าหลังการเก็บเกี่ยวของขนุนพันธุ์ทองพลอย การทดลองแบ่งออกเป็น 3 ส่วน ส่วนที่ 1 การแยกเชื้อ จำแนกเชื้อ และการทดสอบการเกิดโรคของเชื้อราสาเหตุโรคจากขนุน ขนุนที่แสดงอาการโรคผลเน่า 83 ตัวอย่าง พบเชื้อราที่เป็นสาเหตุ 3 สกุล ได้แก่ *Colletotrichum* sp. (44.6%), *Pestalotiopsis* sp. (2.4%) และ *Lasiodiplodia theobromae* (53%) การทดสอบการก่อโรคพบว่า *L. theobromae* เป็นเชื้อก่อโรคหลักที่ทำให้เกิดโรคผลไม้เน่า ส่วนที่ 2 การประเมินผลของโอโซนต่อการเติบโตของเส้นใยเชื้อรา *L. theobromae* ในหลอดทดลอง นำเส้นใยเชื้อราที่เพาะเลี้ยงใน PDA มารมด้วยโอโซน 350 ppm ที่เวลาการรมที่แตกต่างกัน: 0 (กลุ่มควบคุม), 30, 45 และ 60 นาที พบว่าโอโซนสามารถยับยั้งการเจริญของเส้นใยได้เพิ่มขึ้นอย่างมีนัยสำคัญ เมื่อระยะเวลาการรมเพิ่มขึ้น ส่วนที่ 3 ผลของการรมโอโซน เป็นเวลา 60 นาที ต่อการควบคุมโรคผลเน่าในขนุนที่ปลูกด้วยเชื้อรา *L. theobromae* ในระหว่างเก็บรักษาที่ 25 °C เป็นเวลา 7 วัน ผลการวิจัยพบว่าการบำบัดด้วยโอโซนสามารถลดอาการโรคผลเน่าได้ (2.50 เท่า) เมื่อเทียบกับกลุ่มควบคุม และให้ผลลัพธ์ใกล้เคียงกับการใช้สารฆ่าเชื้อรา Prochloraz ที่ความเข้มข้น 150 ppm (2.52 เท่า) ดังนั้น ผลที่ได้จึงแสดงให้เห็นว่าการบำบัดด้วยโอโซนที่ 350 ppm เป็นเวลา 60 นาที สามารถควบคุมโรคผลเน่าในขนุนหลังการเก็บเกี่ยวได้

คำสำคัญ: ขนุน โรคผลเน่า การรมโอโซน

Abstract

The effect of ozone treatment on fruit rot disease control in harvested jackfruits cv. Thong ploy was investigated. The experiments were separated into three parts. First, the isolation and identification of fungal pathogens in jackfruit were observed. Three genera of fungal pathogens that cause fruit rot disease are *Colletotrichum sp*. (44.6%), *Pestalotiopsis sp*. (2.4%), and *Lasiodiplodia theobromae* (53%), from a total of 83 samples. Pathogenicity tests showed that *L. theobromae* was the primary pathogen that caused fruit rot disease. Second, the antifungal effect of ozone treatment on the mycelium growth of *L. theobromae* was evaluated *in vitro*. Fungal mycelium cultured in PDA was treated with 350 ppm ozone at different exposure times: 0 (control), 30, 45, and 60 min. The inhibitory effect of ozone on mycelium growth significantly increased along with the increase in exposure time. In the third part, the effect of ozone fumigation for 60 min on fruit rot disease control in jackfruit inoculated with *L. theobromae* was observed during storage at 25 °C for 7 days. The result showed that ozone treatment could reduce fruit rot disease (2.50-fold) compared to the control, and it displayed similar results to using the fungicide Prochloraz at 150 ppm (2.52-fold). Therefore, the results imply that ozone treatment at 350 ppm for 60 min can potentially control fruit rot disease in harvested jackfruits.

Keywords: jackfruit, fruit rot disease, ozone treatment

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Introduction

The jackfruit (*Artocarpus heterophyllus*) is the largest edible fruit and belongs to the Moraceae family. It is native to Southeast Asia and is popular in several tropical and sub-tropical countries (Hossain and Haq, 2006). Postharvest quality deterioration in jackfruit is commonly caused by fruit rot and chilling injury (Kaur *et al*., 2024). There is limited research available on postharvest disease control in jackfruit even there are no information on the fungal pathogens caused of the fruit rot diseases in Thailand.

Ozone is a well-known strong oxidizing agent that is generally safe to use. It has been used by the food industry as an antimicrobial agent for many years due to its rapid decomposition into harmless oxygen, leaving no residues in food or the environment (Sarron *et al*., 2021). Ozone has been tested as a postharvest treatment against postharvest diseases in different operative conditions over the past few decades, such as papaya (Ong and Ali, 2015), citrus (Garcia-Martin *et al*., 2018), and mango (Bambalele *et al*., 2023). Based on the information above, it can be inferred that ozone treatment can potentially control postharvest disease caused by pathogenic fungi. Therefore, this study aimed to investigate the types of pathogenic fungi that infect harvested jackfruit in Thailand and use of safe compounds, such as ozone, to control fruit rot disease of harvested jackfruit.

Materials and Methods

In this study, jackfruits cv. Thong Ploy was harvested at 85% maturity from an orchard located in Rayong province between April 2023 and January 2024. These experiments were divided into three experiments. In the first experiment, fungi isolation, identification, and pathogenicity tests were conducted. Fungal pathogens were isolated from 83 infected peel samples from jackfruits using the tissue transplanting technique method. The purified fungal isolates were then identified the genus under the compound microscope. The fungal isolates were confirmed their pathogenicity using the Koch Postulates Method. The disease symptom on the inoculated fruit was observed at the wound site and compared with the symptoms of the disease found in the original isolated sample.

The second experiment, the antifungal effect of ozone treatment on the mycelium growth of selected fungal pathogen (*L. theobromae*, which is the major causal fruit rot disease in harvested jackfruit), was evaluated in *in vitro* test condition to find the optimum concentration and time exposure of ozone on mycelial growth inhibition of *L. theobromae*. A mycelia disc (∅ 5 mm) of *L. theobromae* culture (five days old culture) was placed into the center of a PDA plate. All plate samples were fumigated with 325-350 ppm ozone gas for 0 (control), 30, 45, and 60 min. Ozone gas was generated by an ozone generator, model CCA-5G-W, and ozone concentration was measured with an ozone detector (WASP-XM-E-O_3 (maximum dose 5,000 ppm). After ozone treatment, the petri dishes of all treatments were incubated at ambient temperature (25±2 ^{o}C). The diameter of mycelium radial growth on the surface of the PDA medium was observed every day for 3 days by measuring with a ruler until the observed the fungal growth in the control reached the edge of the petri dish. The mycelial growth rate of fungi in each treatment was calculated and expressed as the percentage.

The third experiment, the effect of ozone on fruit rot disease control in jackfruit inoculated with *L. theobromae* was investigated. The jackfruits were wounded with a sterilized knife and sequentially inoculated with the mycelia disc (∅ 5 mm) of *L. theobromae*. All inoculated jackfruits were incubated at ambient temperature (25±2 ^{o}C) with 90-95% humidity for 12 hr. Afterward, the fruit samples were divided into 3 treatments: the control, 150 ppm prochloraz (fungicide) as a positive control, and 320-350 ppm ozone treatment for 60 min (best treatment from the 2nd experiment). The diameter of fruit rot lesion on jackfruit was evaluated after treatment at room temperature for 7 days. All experiments were designed as a

Completely Randomized Design (CRD) with 4 replicates per treatment, then analyzed by analysis of variance using SAS. A completely randomized design and the least significant differences were used to evaluate significant differences between mean values ($P < 0.05$).

Results and Discussion

Isolation, identification and pathogenicity test

The eighty-three fungal isolation was obtained from jackfruit. The results showed that three distinct fungal genus were identified: *Colletotrichum* sp., *Pestalotiopsis* sp., and *Lasiodiplodia theobromae* (Fig. 1B), with frequencies of 44.6%, 2.4%, and 53%, respectively (Fig. 1A). The pathogenicity tests of three fungal pathogens were conducted. Among of these fungal pathogens, *L. theobromae* showed the most severe fruit rot symptom and produced rapid growth on the fruit compared to other two fungi. Thus, *L. theobromae* can be identified as the main fungal pathogen responsible for causing fruit rot disease in harvested jackfruit cv. Thong Ploy in Thailand. A similar result was observed by Adikaram *et al*. (2020) in Sri Lanka, where they identified *L. theobromae* as the primary pathogen responsible for a fruit rot disease in ripe jackfruits. In contrast, Coronado-Partida *et al*. (2023) in Mexico reported that *Rhizopus stolonifer* is the predominant pathogen causing soft rot disease in harvested jackfruits.

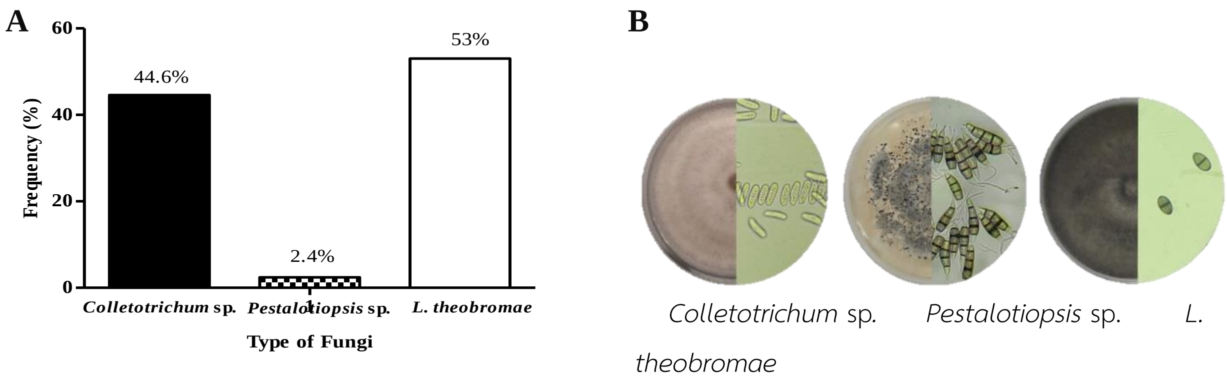

Figure 1. Frequency of fungal pathogens causes of fruit rot disease in jackfruit cv. Thong ploy (A), and illustration of mycelium and spore of *Colletotrichum* sp., *Pestalotiopsis* sp., and *L. theobromae* (B).

The antifungal effect of ozone treatment on the mycelium growth of *L. theobromae*

The mycelial growth rate (%) of *L. theobromae* exhibited variability across different treatment groups (Fig. 2A and 2B). The ozone treatment, when the pathogenic fungi was subjected to all time exposures, exhibited a significantly ($p < 0.05$) lower mycelial growth compared to the control. The control exhibited complete mycelial growth (100%) by day 2, while the mycelium growth in 325-350 ppm ozone treatment for 60 min was the lowest (6.40%) followed by 45 min (46.62%) and 30 min (59.49%), respectively. A similar study demonstrated by Savi and Scussel (2014) that treatment with gaseous ozone at 60 µmol/mol for 120 min had the highest percentage of mycelial growth inhibition of *F. graminearum* and *P. citrinum,* the casual agents of fruit rot in kiwi fruit, compared to treatments of 40, 60, and 90 min. Ozone disinfection mechanisms can be related to fungi cell metabolism alterations, which lead to apoptosis and oxidative stress, and have proved to be effective in controlling toxigenic fungal development (Savi and Scussel, 2014). Guzel-Seydim *et al.* (2004) also reported that ozone can degrade unsaturated lipids of the microbial cell membrane, led to membrane leakage and cell breakdown. Consequently, the 325-350 ppm ozone treatment for 60 min was selected for the third experiment.

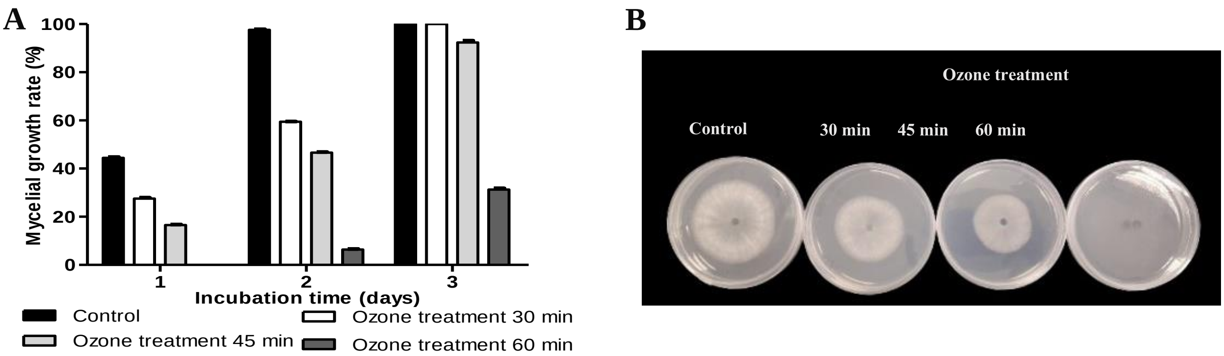

Figure 2 Effect of ozone gas at 325-350 ppm for 0 (control), 30, 45, and 60 min on mycelial growth of *L. theobromae* during incubation at room temperature (A) and the illustration of *L. theobromae* mycelium of PDA medium after exposing with ozone gas for 2 days (B).

The effect of ozone treatment on fruit rot disease control in jackfruit inoculated with *L. theobromae*

As a broad-spectrum fungicide, ozone has been widely used to manage postharvest decay in fruit and vegetables by affecting the growth of pathogenic fungi. This present study showed that the fungal mycelium presented in non-ozone treated fruits (control) after three days of storage, and then expanded rapidly until day 7 of storage (Fig. 3A). The disease lession of the control fruit on day 7 was 26.3 cm, whereas the disease severity of *L. theobromae* in jackfruit was significantly inhibited after treatment with the 325-350 ppm ozone for 60 min (10.5 cm) and 150 ppm prochloraz (10.4 cm) respectively (Figure 3B). This result demonstrates that ozone can effectively control fruit rot disease by reducing the fungal contamination of harvested jackfruit. The result is consistent with a report by Ong and Ali (2015), who found that ozone treatment (2.5 μL/L for 24 hr) significantly inhibited the disease incidence of anthracnose caused by *C. gloeosporioides* (3.4-fold). Similarly, Li *et al*. (2022) reported that 2 mg/L ozone treatment for 2 min significantly inhibited the development of potato dry rot in tuber slices inoculated with *F. sulphureum* (67.2%). The inhibition mechanism of ozone is attributed to its capacity to affect the growth of pathogenic fungi by destroying fungal cell membrane structure (Li *et al*., 2022).

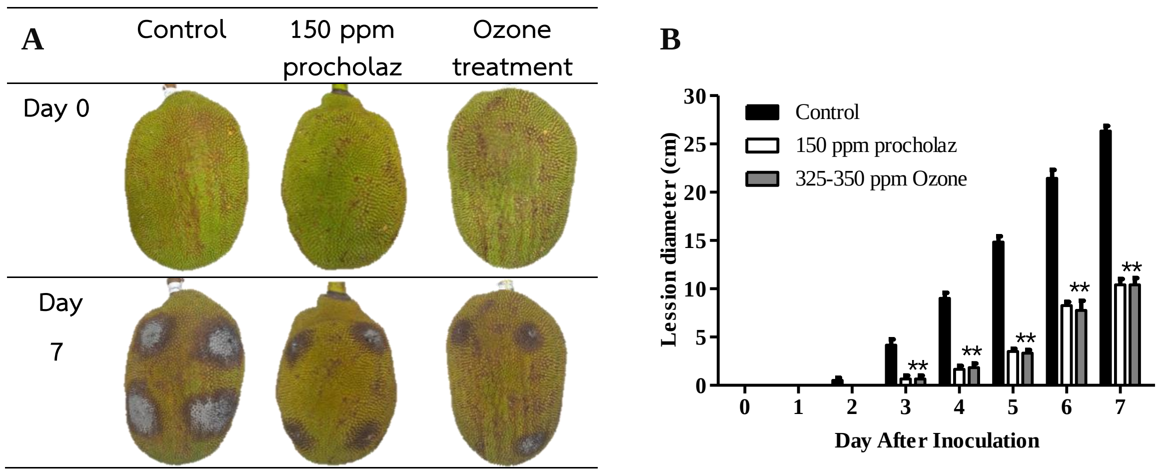

Figure 2. Illustration of fruit rot disease (A) and the lesion diameter of fruit rot symptom in jackfruit inoculated with *L. theobromae* after treatment with 325-350 ppm ozone for 60 min, compared with 150 ppm prochloraz (fungicide), and the untreated control fruit (B) during storage at ambient temperature for 7 days.

Conclusion

This study identified three fungal pathogens that infected harvested jackfruit in Thailand: *Collectotrichum* sp., *Pestalotiopsis* sp., and *L. theobromae*. *L. theobromae* is considered the main pathogen responsible for fruit rot disease of jackfruit in Thailand. The ozone treatment at 325-350 ppm for 60 min was found to be the most effective concentration for inhibiting the mycelium growth of *L. theobromae* and fruit rot disease in harvested jackfruit cv. Thong ploy.

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