การประยุกต์ใช้ไคโตซานและคาเฟอีนต่อการงอกของเมล็ดข้าวไรซ์เบอรี่
APPLICATIONS OF CHITOSAN AND CAFFEINE IN RICEBERRY SEED GERMINATION

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บทคัดย่อ

งานวิจัยนี้ศึกษาผลของไคโตซานที่เตรียมได้จากไคตินจากเปลือกกุ้งและผลของคาเฟอีนจากกากเมล็ดกาแฟต่อการงอกและการเจริญต้นอ่อนของข้าวไรซ์เบอรี่ โดยนำเมล็ดข้าวมาแช่ในสารละลายคาเฟอีนความเข้มข้นร้อยละ 1, 3 และ 5 โดยมวลต่อปริมาตร และสารละลายไคโตซานความเข้มข้นร้อยละ 0.10, 0.30 และ 0.50 โดยมวลต่อปริมาตร เป็นเวลา 7 วัน พบว่าสารละลายคาเฟอีนทุกความเข้มข้นและสารละลายไคโตซานความเข้มข้นร้อยละ 0.10 โดยมวลต่อปริมาตร สามารถเพิ่มการงอกของเมล็ดข้าวได้มากกว่ากลุ่มควบคุมที่ใช้น้ำ ในขณะที่สารละลายไคโตซานความเข้มข้นร้อยละ 0.30 และ 0.50 โดยมวลต่อปริมาตร พบว่าเมล็ดข้าวไม่งอก เมื่อศึกษาการเจริญของต้นอ่อนโดยนำเมล็ดข้าวปลูกลงดินในกระถางและรดต้นข้าวด้วยสารละลายคาเฟอีนและสารละลายไคโตซานแทนการใช้ปุ๋ยและสารเคมี เปรียบเทียบกับกลุ่มควบคุมที่ใช้น้ำ พบว่าความสูงของต้นอ่อนข้าวไรซ์เบอรี่ ที่รดด้วยน้ำกับข้าวไรซ์เบอรี่ที่รดด้วยสารละลายคาเฟอีนใน 10 วันแรกนั้น มีระดับใกล้เคียงกัน แต่ที่ 30 วันต้นข้าวที่รดด้วยคาเฟอีนเข้มข้นร้อยละ 3 และ 5 โดยมวลต่อปริมาตรมีการเจริญเติบโตดีกว่าการรดด้วยน้ำมากถึงร้อยละ 15-30 ส่วนต้นข้าวไรซ์เบอรี่ที่รดด้วยไคโตซานความเข้มข้นร้อยละ 0.10 โดยมวลต่อปริมาตร จะมีการแตกรากและการเจริญเติบโตของลำต้นใกล้เคียงกับการรดด้วยน้ำ ในขณะที่ความเข้มข้นร้อยละ 0.30 และ 0.50 โดยมวลต่อปริมาตร ต้นข้าวไรซ์เบอรี่ที่ใช้แช่ไม่มีการแตกราก เมื่อปลูกลงดินจึงไม่มีการเจริญเติบโต จากผลการวิจัยนี้สามารถนำไปต่อยอดสู่การปลูกข้าวไรซ์เบอรี่แบบเกษตรอินทรีย์ที่สามารถเพิ่มการงอกของเมล็ดข้าวและช่วยให้ต้นอ่อนข้าวสูงขึ้น

คำสำคัญ: ไคโตซาน คาเฟอีน ข้าวไรซ์เบอรี่

Abstract

This research paper aims to study the effect of chitosan preparation from shrimp shell chitin and the effect of caffeine from coffee grounds on seed germination and seedling growth of riceberry. The experiment was performed with seed soaking 1%, 3% and 5% w/v in caffeine solution and 0.10, 0.30 and 0.50 % w/v in chitosan solution for 7 days. The results showed that all concentration of caffeine solution and 0.10% of chitosan solution stimulated seed germination over the control water-soaked rice whereas application by 0.30% and 0.50% w/v of chitosan solution did not show the effect of seed germination. A study of seedling growth was conducted by sowing seeds in pots and watered with caffeine and chitosan solutions instead of chemical fertilizers and pesticides. When compared to water-soaked rice after 10 days treatment, the results did not show significant differences of a height of seedling growth of riceberry watered with caffeine. However, the seedlings grow 15-30% in height when performed with 3% and 5% w/v of caffeine solution after 30 days of treatment comparing with the control while 0.10 % of chitosan solution showed no significant differences in stimulating root growth and seedling growth when compared to the control. Whereas 0.30 and 0.50 % w/v of chitosan solution did not affect the root growth so it cannot grow when sowing seeds in soil. This study can be further developed in rice organic farming to increase in seed germination and seedling growth.

Keywords: chitosan, caffeine, riceberry rice

Introduction

Nowadays, organic agriculture is one of the most dynamic and rapidly-growing sectors of the global food industry (Pornpratansombat et al., 2011). Due to the use of chemical fertilizers and pesticides in conventional agriculture, this process causes major issues concerning farmers and customers' health problem, soil erosion and financial considerations. In Thailand, the adoption of organic rice farming is one of several approaches in sustainable agriculture because of the need for commercial viability. In order to meet current and future needs of a growing world population, it's necessary to provide solutions for solving the major problems in conventional agriculture. As a means to adopt the Royal Initiative of the 'Philosophy of Sufficiency Economy' to promote sustainable agriculture, this research aims to study residues and waste from the food industries which includes frozen shrimps and coffee grounds application for organic rice farming.

Chitosan is a natural biopolymer modified from chitin. Chitin and chitosan are copolymers found in nature which are the main components of shrimp, crab, snail shells, squid pens and cell walls of some fungi (Dutta et al., 2004). They are inherent to have specific properties of being environmentally friendly and easily degradable. There are abundant raw materials for chitosan production as Thailand is the world-leading exporter of frozen shrimps (Boonlertnirun et al., 2008). Chitosan has a wide scope of application in food industry and biomedical agriculture. In agriculture, chitosan is the one of biomaterials used for increasing plant yield and decreasing plant disease. Chitosan regulates the immune system of plants and induces the excretion of resistant enzymes. Chitosan is not only activates the cells, but also control its disease and develop insects resistance (Boonlertnirun et al., 2008; Mahdavi et al., 2013).

Caffeine (1, 3, 7-trimethylxanthine) found in coffee and tea stimulates the central nervous, respiratory and cardiac system (Khursheed et al., 2009). According to coffee consumption grows in Thailand, the residue of ground coffee has been increasing. Coffee grounds, which are the coffee residue after brewing, are considered solid waste and normally dumped into landfills. Coffee in landfills takes up space until they are degraded. They also obtain energy by oxidizing organic material to get adequate oxygen in order to be degraded. This oxidation process heats up the compost pile to ferment and spontaneously combust (Hall & Gu, 2014). Previous research has suggested that coffee grounds can be used as soil additives (Yen et al., 2005), metal absorbents (Utomo & Hunter, 2006) and weed control (Sciarappa et al., 2008). Chitosan and coffee grounds are biomaterials and low cost by-products, they could be an option for the organic agriculture. The aim of this study is to investigate the effect of chitosan extracted from shrimp shells and caffeine extracted from coffee grounds on rice seed germination and seedling growth. In this research, we used riceberry rice; which is rich in antioxidants that help our immune system stay healthy, to suggest widespread cultivation in Thailand.

Materials and methods

Chitosan preparation:

Fresh shrimp shells (*Litopenaeus vannamei* or Whiteleg shrimp) were collected from local market and seafood restaurant at Bandu Sub District Municipality. The collected shrimp waste were washed with water, dried and crushed with mortar pestle. Crushed shrimp were kept in a zip-lock plastic bag at ambient temperature in a desiccator for 24 h by the method of chitosan extraction from chitin heating temperature of 65-100°C for 3-4 h compared to the one without heating solution. Chitosan was prepared from isolated chitin by the following three steps: 1) deproteinization; crushed shrimp were placed in two of 1000 ml beakers, one is soaked in boiling 4% w/v NaOH for 3 h and another is soaked in ambient temperature 4% w/v NaOH for 24 hours, after that allowed them to cool for 30 minutes. The residue were washed and soaked in water until neutral pH. 2) demineralization; the crushed shrimp shell is demineralized using 4% v/v HCl with boiling 65°C for 3 hours and without heating by soaked in 4% v/v HCl for 24 hours. The remaining chitin is washed in water and drained. Then the chitin is crushed into small sized pieces 0.5-5 mm. 3) deacetylation; the chitin was further converted into chitosan in this process. Removal of acetyl groups from chitin used 40% w/v NaOH at 100°C for 3 hours on a hotplate and another is soaked in 40% w/v NaOH at room temperature for 48 hours. The samples are washed and filtered retain the solid matter, which is the chitosan. The samples were dried at 110°C for 5 hours. Stock chitosan solution was prepared by dissolving chitosan in 2% acetic acid solution.

Caffeine preparation:

Coffee grounds (Coffea arabica L.) were collected from coffee shop at Ban Du sub-district, Chiang Rai province. Stock coffee solution was developed by mixing coffee grounds with water at the concentration of 1%, 3% and 5% w/v and stirring for 24 hours on a hotplate stirrer.

Seed treatment and germination experiment:

Riceberry seeds were soaked in water for 12 hours. 20 seeds were selected under a condition. After soaking, the first group of seed was submerged in different concentrations of chitosan solutions (0.10%, 0.30% and 0.50% dissolved in 2% acetic acid solution) for 3 hours at 25°C. The second group of seeds was submerged in different concentrations of caffeine solution (1%, 3% and 5% dissolved in water) for 3 hours at 25°C. The third group of seed, the control one, was submerged in water for 3 hours at 25°C. Three replications of 20-seeds under different conditions were placed in soil in the 9-cm glass petri-dish and kept all of dishes at room temperature for 7 days. The number of total germinated seeds and length of root were measured. At the same time, three replications of 20-seeds under different conditions were planted in soil in 1-liter plastic cups and kept all of cups outdoor. The first group was watered with 0.10% chitosan solution. The second group was watered with 1%, 3% and 5% caffeine solutions and the third group was watered with water. Germination rate and seedling growth as indicated by root and shoot length were measured for 30 days.

Results and discussion

The results of germinated seeds indicated that the seeds pretreated with caffeine and 0.10% of chitosan significantly affected the germination percentage more than the water treatment. All concentrations of caffeine solution and 0.10% of chitosan solution showed the highest percentage of seed germination (100%) whereas the control had only 80% of seed germination. However, 0.30% and 0.50% of chitosan solution was completely inhibited seed germination. After 7 days, the root length was measured. The results showed that caffeine pretreatment significantly increased germination, especially in 5% of caffeine-soaked. The seeds soaked with 5% of caffeine showed the longest root (2.78 cm) while root length received from the control was only 1.36 cm as shown in Table 1

Table 1 Root length of germinated seeds on 7th day.

types of solution	root length (cm)
1. water	1.36 ±0.50
2. 0.10 %w/v chitosan	1.70 ±0.20
3. 0.30 %w/v chitosan	0.00
4. 0.50 %w/v chitosan	0.00
5. 1.00%w/v caffeine	1.80 ±0.30
6. 3.00%w/v caffeine	1.75 ±0.30
7. 3.00%w/v caffeine	2.78 ±0.20

The height of riceberry seedling after 30 day of sowing and mature plant height were 21.25, 23.50, 23.57, 30.33 and 23.75 cm in control, 0.1% chitosan, 1%, 3% and 5% caffeine watering treatment, respectively. Rice seedling soaked and watered with 3% of caffeine showed the highest of mature plant with 30.33 cm more than the control group with 21.25 cm, as shown in Table 2

Table 2 Riceberry seedling height (cm).

types of solution	rice seedling height (cm)
1. water	21.25 ±0.60
2. 0.10 %w/v chitosan	23.50 ±0.40
3. 0.30 %w/v chitosan	0.00
4. 0.50 %w/v chitosan	0.00
5. 1.00%w/v caffeine	23.57 ±0.30
6. 3.00%w/v caffeine	30.33 ±0.20
7. 3.00%w/v caffeine	23.75 ±0.50

Riceberry seed pretreatment with different concentrations of caffeine resulted in increasing germination percentage, root length and seedling height which is higher than the control group. The optimum concentration of caffeine was 3% w/v of coffee ground stock in water. Caffeine treatment on seeds and watering on rice seedling plant could increase the rice seed germination rate and seedling growth due to its components. Allelopathic compounds are the potential chemicals components exist in almost all plants and most tissues, such as caffeine in coffee seed, can positively or negatively affect growth vegetation (Maryam & Mansour, 2007). The benefit of using coffee grounds as a fertilizer is its addition in organic material to soil, which improves drainage, water retention and attraction of water to the soil. It creates a nitrogen-rich soil effect because of its compounds of nitrogen as shown in coffee chemical formula is $C_8H_{10}N_4O_2$. Moreover, coffee ground increase buffering capacity against leaching of nitrates and changes in pH, increase biological activity and resilience against pathogens (Sarah Hardgrove, 2015).

In the case of chitosan, we found that 0.10% w/v of chitosan increased seed germination and seedling growth more than the control group. Chitosan promotes plant growth through increasing the availability and uptake of water in plants and essential nutrients through adjusting cell osmotic pressure (Guan et al., 2009). The use of chitosan to benefit rice seed priming can enhance seed germination performance, however in higher concentration of chitosan showed an inhibitory effect to seed growth. It can assume that seed coated with higher chitosan level creates thick chitosan biopolymer films (Marcia Z.B. et.al, 2015) around seed and it may hinder permeability of oxygen which is essential during seed germination. The in-depth study of the effect of higher concentration of chitosan on seed germination has prepared to further study.

Conclusion and suggestion

Chitosan which extracted from shrimp shells and caffeine which extracted from coffee grounds showed the effectiveness of riceberry seed germination and seedling growth. 1%, 3% and 5%w/v of caffeine can increase the germination more than the control group. In the study of chitosan, 0.10%w/v of chitosan can increase seed germination, however the higher concentration of 0.03 and 0.05%w/v did not show germination of seeds. The effect of watering with chitosan and caffeine solution on seedling growth showed that the groups of 3% and 5% w/v of caffeine have higher seedling growth than the control group 15-30% in 30 days. However, further studies are needed to explicitly elucidate the mechanism of action to explain the effects of caffeine and chitosan in plant growth. Chitosan and caffeine are biomaterials and low-cost by-products. Finally, the recommendation of the findings in this study is to investigate in the rice farming in order to promote rice organic farming.

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