Agricultural Sci. J. 47 : 3 (Suppl.) : 35-38 (2016) ว. วิทย. กษ. 47 : 3 (พิเศษ) : 35-38 (2559)

การประยุกต์ใช้สารเรืองแสงเพื่อการทดสอบสารพิษตกค้างในผัก

Applications of Fluorescence to Test Pesticide Residues in Vegetables

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บทคัดย่อ

ปัจจุบันผู้บริโภคมีความตระหนักด้านสารพิษตกค้างในผลิตผลเพิ่มขึ้น มีการศึกษาจำนวนมากเกี่ยวกับการตรวจสารป้องกันกำจัดศัตรูพืชตกค้างโดยใช้วิธีการต่างๆกัน ซึ่งแต่ละวิธีมีค่าใช้จ่ายสูง ทำได้ยาก และใช้เวลานาน ดังนั้นการศึกษาครั้งนี้จึงทดสอบการใช้สารเรืองแสง CMU1 (an aromatic fluorescence substance) เพื่อใช้เป็นสารบ่งชี้การตกค้างของสารป้องกันกำจัดศัตรูพืชในพืชผัก โดยการฉีดพ่นใบผักคะน้าจีนในสภาพแปลงปลูกด้วยชุดการทดลองดังนี้ 1) น้ำกลั่น (ชุดควบคุม), 2) CMU1 ความเข้มข้น 2.5 mg/l, 3) สารละลาย 2% Fe–EDTA, 4) สาร chlorpyrifos ความเข้มข้น1 mg/l (สารป้องกันกำจัดแมลง), 5) สารละลาย CMU1 ความเข้มข้น 2.5 mg/l + สาร chlorpyrifos ความเข้มข้น 1 mg/l , 6) สารละลาย 2% Fe–EDTA + สาร chlorpyrifos ความเข้มข้น 1 mg/l และ 7) สาร CMU1 ความเข้มข้น 2.5 mg/l + สาร 2%Fe–EDTA + สาร chlorpyrifos 1 mg/l ตรวจสอบการเรืองแสงของสารละลายใบใบผักภายใต้แสงUV ในกล่องมืดทุกวันเป็นเวลา 14 วัน พบว่า chlorpyrifos และ Fe-EDTA ไม่สามารถเรืองแสงได้ ส่วนสารCMU1 ทั้งที่มี และไม่มี Fe-EDTA สามารถเรืองแสงสีฟ้าได้นาน 7 วัน ภายหลังการฉีดพ่น ความเข้มของแสงเรืองสีฟ้าลดลงตามจำนวนวันภายหลังการฉีดพ่น จึงพอสรุปได้ว่า CMU1 เป็นสารที่มีศักยภาพในการพัฒนาเพื่อใช้เป็นดัชนีในการบ่งชี้การตกค้างของสารป้องกันกำจัดศัตรูพืช

คำสำคัญ : การตกค้างของยาฆ่าแมลง, ตัวชี้วัดการตกค้างของยาฆ่าแมลง, สารเรืองแสง

Abstract

Presently, pesticide residue awareness is rising among consumers. There were many studies in pesticide residue detections in fruit and vegetables with many methods, but they were not easy and took a long time to do. Therefore, this study tried to use CMU1, an aromatic fluorescence substance, as an indicator of pesticides residue in vegetables. The experiment was as follows: (1) distilled water (control), (2) CMU1 2.5 mg/l, (3) Fe–EDTA 2%, (4) chlorpyrifos (insecticide) 1 mg/l, (5) CMU1 2.5 mg/l + chlorpyrifos 1 mg/l, (6) Fe–EDTA 2% + chlorpyrifos 1 mg/l, and (7) CMU1 2.5 mg/l + Fe–EDTA 2% + chlorpyrifos 1 mg/l. All solutions were sprayed on Chinese kale leaves. The fluorescence was investigated under UV light in an UV box (a darken box) every day for 8 days. It was found that CMU1 with/without Fe-EDTA mixed with pesticide could emit the blue fluorescence light under UV light in an UV box for 7 days. The intensity of the blue fluorescence light was decreased with time after spraying. It could be inferred that CMU1 had potential to be used as a pesticide residues index.

Keywords: pesticide residue, pesticide residue index, fluorescence substances

Introduction

Pesticides are chemicals that help to protect plants against molds, fungi, rodents, and insects etc. They help to prevent crop loss and, potentially, human disease. Numerous pesticides are widely used all over the world. The most common categories of pesticides imported into Thailand are herbicides, followed by insecticides and fungicides (Sapbamrer and Nata, 2014). Presently, pesticide residue awareness is rising among consumers.

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There were many studies in pesticide residue detections in fruit and vegetables with many methods but they were costly, difficulty and took a long time to do. (Wanwimolruk *et al*., 2015)

In Thailand, a number of popular Thai dishes have Chinese kale (*Brassica oleracea*) as a main ingredient; and in some dishes, Chinese kale is consumed fresh, without cooking. This has potential toxic to human if the Chinese kale has pesticide residue over the MRL (Codex Alimentarius Commission, 2015).

CMU1 is an aromatic fluorescence substance that exhibits chemiluminescence, with a striking blue fluorescence, when mixed with an appropriate oxidizing agent. CMU1 is a white-to-pale-yellow crystalline solid. After activation by ultraviolet light, CMU1 will emit a blue fluorescence that can be seen in a dark room. The blue fluorescence will be faded by the time and temperature. In this study, the potential to use CMU 1 as a pesticide index was revealed.

Material and Methods

CMU1 at the concentration of 2.5 mg/l was prepared by mixing 0.05 g CMU1 in 10 ml distilled water with 0.5 g NaOH and then added with 3% H_2O_2 10 ml. Chlorpyrifos, an organophosphate insecticide; Chlorified40™ (Sigma Agrochemical, Bangkok, Thailand), was obtained from the commercial insecticide which sold in an agrochemical shop. 2% Fe-EDTA solution was obtained by dissolved Fe-EDTA 2 g with distilled water 100 ml. In first experiment, the experimental design was CRD, 4 treatments with 4 replications. Sixteen 1.5 ml microcentrifuge tubes were filled with 0.7 ml concentrated chlorpyrifos solution. The filled tubes were added with 1) 2.5 ml/l CMU1 0.3 ml, 2) 2% Fe–EDTA 0.3 ml and 3) 2.5 ml/l CMU10.15 ml, 2% Fe–EDTA 0.15 ml. The non-added tubes were used as 4) control. All of the tubes were took to determine their fluorescence properties under UV light in a UV box, a darken box, every other day for 14 days. The intensity of the blue fluorescence was scored 1 – 4 (Table 1). In second experiment, The CRD was employed. There were 7 treatments with 4 replications, 1) distilled water (control); 2) CMU1 2.5 mg/l; 3) Fe–EDTA 2%; 4) chlorpyrifos 1 mg/l; 5) CMU1 2.5 mg/l + chlorpyrifos 1 mg/l; 6) Fe–EDTA 2% + chlorpyrifos 1 mg/l; and 7) CMU1 2.5 mg/l + Fe–EDTA 2% + chlorpyrifos 1 mg/l. All of the solutions were sprayed on Chinese kale leaves which grew in the open field. The leaves were harvested and check for fluorescence properties every day for 8 days under UV light in the UV box (The obtained data will be analyzed fluorescence by scoring as 4 point Hedonic scale).

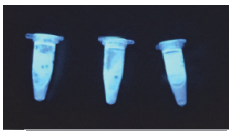
Result and Discussion

After mixing chlorpyrifos with CMU1 with or without Fe–EDTA in microcentrifuge tubes, the color of the insecticide did not change in all treatments. Our bare eyes could not discriminate the treatments into different groups (Fig. 1A) in this study period, 14 days. It was found that chlorpyrifos and Fe–EDTA could not emit fluorescence under UV light. In another hand, the treatments with CMU1 with or without Fe–EDTA could emit blue fluorescence under UV light and the blue fluorescence did not faded in all of this study period (Fig. 1B). After spraying the leaves with the treatment solutions, the leaves did not show any sign of being sprayed. The sprayed and non-spray leaves did not difference. The chlorpyrifos with or without Fe-EDTA sprayed leaves did not emit fluorescence under sun and UV light. All of the CMU1 treatments could emit blue fluorescence under the UV light (Fig.2) for 7 days. The intensity of the blue fluorescence was reduced by the time after spraying. The intensity of the fluorescence light was scored 4 for 3 days after spraying then the fluorescence intensity scores were decreased gradually until they lost their fluorescence properties after spraying for 7 days. CMU 1 did not cause any damage to Chinese kale leaves.

Table. 1 Scoring Scale for blue light intensity which emitted by CMU1 under ultraviolet light in a dark box

Level of fluorescence under UV light	Score	Blue fluorescence light intensity
	1	Non-fluorescence
	2	Low fluorescence light
	3	Medium fluorescence light
	4	High fluorescence light

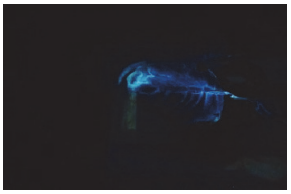
A.



B.

C.

Fig. 1 (A) Chlorpyrifos with CMU 1 with/without Fe–EDTA under sun light (non-fluorescence, scoring of the fluorescence light intensity was 1 point).
(B) Chlorpyrifos with CMU 1 with/without Fe–EDTA emitted blue fluorescence under the UV light (high fluorescence light intensity, scoring of the fluorescence light intensity was 4 point).
(C) Chlorpyrifos without CMU1 did not emit under the UV light (non-fluorescence, scoring of the fluorescence light intensity was 1 point).



A.

B.

Fig. 2 (A) Chinese kale leaves sprayed with chlorpyrifos and CMU 1 emitted blue fluorescence under the UV light (scoring of fluorescence is 4 point).
(B) Chinese kale leaves sprayed with chlorpyrifos did not emit under the UV light.

This study showed that CMU1 has a high potential to be developed as a pesticide residue index because it could stable for a long time when mixing with the concentrate pesticide before using and it could be degrade by time and sun light along with the pesticide.

Conclusion

CMU 1 did not cause injury on Chinese kale leaves. CMU1 has a high potential to be developed as a pesticide residue index.

Acknowledgement

The authors gratefully acknowledge financial support from Agricultural Research Development Agency (Public Organization), Science and Technology Park Chiang Mai University, Postharvest Technology Research Institute, Chiang Mai University, Mae Hia Agricultural Research, Demonstrative and Training Center.

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