

Use of Polylactic Acid Added with Calcium Oxide and 3-Iodo-2-Propynyl Butyl Carbamate as an Antifungal Agent and Bio-degradable Soil Remediator

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Abstract

This work studied the use of polylactic acid added with calcium oxide (CaO) and 3-Iodo-2-propynyl butyl carbamate (IPBC) for agricultural applications. CaO content of 30 pph was fixed, while IPBC contents were varied at 0, 2.5, 5.0, 7.5 and 10.0 ppm in the polymer matrix. All ingredients were mixed by the extrusion process for receiving the PLA, PLA/CaO, and PLA/CaO/IPBC granules. The pH value, antifungal activities of *Phytophthora parasitica*, tomato growth, and disposal degradation were also investigated. The experimental results indicated that the acidic condition of the PLA granules increased with increasing IPBC contents but was compromised by CaO. An IPBC was an effective antifungal agent. It was also promoted and activated by CaO. Growth of *P. parasitica* can be inhibited by CaO synergized IPBC for the PLA/CaO granules with four different IPBC contents and the PLA/CaO granules with the four different IPBC contents were an effective antifungal agent. A PLA/CaO granule with an IPBC content of 2.5 ppm can be developed to compete with an unused condition. A PLA/CaO granule with an IPBC content of 10 ppm was recommended for agricultural applications. A PLA/CaO/IPBC 10 acted as an effective antifungal agent and compostable for soil bioremediation before planting. Over the 45-day experimental period, the maximum percentage of weight loss of a PLA/CaO granule with an IPBC content of 10 ppm was influenced by moisture and initial PLA loading. A PLA/CaO granule with an IPBC content of 10 ppm (47.2%) had a faster degradation rate than a PLA/CaO granule (45.3%) and a neat PLA granule (15.0%).

Keywords: Biodegradable, Polylactic acid, Calcium oxide, Antifungal agent, Soil bioremediation

Introduction

Fungi present an important plant disease that reduces crop yield and price. Studies of the correlation between humidity requirement and fungal growth revealed that the minimum humidity permitting the growth of fungi varied from 70–90% for different fungal species (Block, 1953; Menneer et al., 2022). Some fungi types have been shown to destroy the root, stalk, leaf and raceme of plant organisms. Many types of plant diseases affect growth and subsequently harvest yield (Prescott et al., 1986). For citrus crops, uncontrolled plant disease and fungal pathogenicity from *Penicillium digitatum* (Green fungus) and *P. italicum* (Blue fungus) significantly increase economic loss (Cheng et al., 2020). Phytophthora disease is frequently found in many economic crops in Thailand such as tomatoes (Larousse et al., 2017), durian (Kongtragoul et al., 2021), and rubber tree (Chee, 1969). The roots of the crops can be damaged as well as the above-ground parts; both fruit and leaves. Black fungus on tomato leaves also significantly negatively impacts tomato yield (Hartman & Wang, 1992).

Calcium oxide (CaO) is a product of the carbonation of limestone. It is categorized as basic and is widely used as an agricultural chemical for reducing acidic conditions in soil both before and after plantings (Bast et al., 2011; Radziemska et al., 2019). It also inhibits microbial growth under appropriate humidity conditions (Na-ngam et al., 2004). It has gained much attention worldwide because of its promising agricultural applications. The effect of urea and CaO on *Pseudomonas solanacearum* survival for tomato wilt incidence is to suppress the population and therefore toxicity of *P. solanacearum* by using soil incorporated with CaO (Michel et al., 1997). An addition of CaO enhanced the antibacterial activity of the polypropylene (PP) composites and created low toxicity (Liang et al., 2022). CaO was shown as an eco-friendly natural inorganic antibacterial material. CaO synthesised via an eco-friendly method was a potentially useful chemical in agriculture (Gandhi et al., 2021). It also alleviated and reduced the root-to-shoot transportation from Arsenic (As) toxicity (Nazir et al., 2022). Also, a CaO biocompatible polymeric coating acts against weight loss in cucumbers in postharvest storage of 15 days because of decreasing dehydration (Cid-López et al., 2021).

3-Iodo-2-propynyl butyl carbamate (IPBC) is a fungicide and an antimicrobial agent. It is used as a preservative in adhesives, paints, latex paper coating, plastic, water-based inks, metal working fluids, textiles and numerous consumer products, and as a sapstain control chemical in wood products. It has a role as an environmental contaminant and an antifungal agrochemical. Considering the unaffacting ecosystem, the use of IPBC as a biocidal mixture on endpoint reproduction, and the avoidance behavior of *Folsomia candida* as a soil organism for warning IPBC concentration, it was limited to use for many applications. (Guimarães et al., 2018). Several pieces of research on IPBC were found in polymeric products, namely a biocide as a growth inhibitor of *Aspergillus niger* of linear low-density polyethylene (LLDPE) (Gitchaiwat et al., 2013) and an *A. niger* fungicide of wood/PVC composites (WPVC) through disk diffusion and dry weight techniques. It was found that the most satisfactory growth inhibition of *A. niger* was gained from using an IPBC content of 10,000 ppm (Kositchaiyong et al., 2014). Moreover, the degradation of polymeric materials was retarded by IPBC, both UV and disposal conditions (Kositchaiyong et al., 2014).

Polylactic acid (PLA) is a compostable polymer. It is derived from sustainable natural resources, namely sugar cane, corn, and cassava via polymerization. It was initiated for tissue applications such as tissue scaffolds, medical implants, and internal sutures (Santoro et al., 2016; DeStefano et al., 2020). The outstanding property was recently applied as a degradable material for eco-friendly food and beverage packaging and cutlery (Rhim et al., 2009). Modification of antimicrobial properties of PLA film embedded with silver and chitosan was also studied (Turalija et al., 2016). There were many works that studied the antimicrobial activity of PLA blended with different biocides. Gentamicin sulphate having 0.1 wt% of PLA/PEG solution was susceptible antibacterial on *Staphylococcus aureus* of electrospun PLA/PEG fiber mats (Threepopnatkul et al., 2010). This work was similarly found using triclosan/cyclodextrin complexes (Kayaci et al., 2013). Synthetic silver nanoparticles into PLA via a chemical reduction in a diphasic solvent on an antibacterial characteristic of silver/PLA nanocomposite (Ag/PLA-NC) films, revealed that Ag/PLA-NC films can be used as an antibacterial scaffold for tissue engineering and medical applications (Shameli et al., 2010). Considering the quantitative study of PLA, it found that laundered PLA fibers affected the antibacterial activity of PLA incorporated with a microbicidal agent because of the removal of lactide (Kaźmierczak et al., 2016). However, the addition of silver-substituted zeolite (Zeomic) did not perform antibacterial activities for both neat PLA and wood/PLA due to non-diffusivity of silver in Zeomic, while wood and Zeomic tended to increase the degree of biodegradation of PLA and wood/PLA

materials, whereby the PLA having 10%wt of wood with 1.5%wt of Zeomic had the most satisfactory biodegradation level as a consequence of accelerated hydrolysis degradation from moisture in wood and Zeomic (Praprudivongs & Sombatsompop, 2013). Moreover, wood acted as an antimicrobial promoter for triclosan-filled PLA (Praprudivongs & Sombatsompop, 2012).

In this work, the PLA and PLA/CaO granules with four different IPBC contents were used for studying antifungal activities for *Phytophthora parasitica*. PLA act as a coordinator and host to control the release rate of IPBC into the soil. The disk diffusion and dry weight techniques against *P. parasitica* growth were considered antifungal activities of the PLA and the PLA/CaO granules. Measurement of pH values of the PLA and the PLA/CaO granules were also investigated. The selective PLA and PLA/CaO granules against granulating periods were used for studying both baby and adult tomato growth. Finally, a recommended PLA/CaO granule from selective PLA and PLA/CaO granules was studied for degradation of disposal, as compared with neat PLA and PLA/CaO.

Methods and Materials

Raw material and sample preparation

For the preparation of PLA and PLA/CaO granules incorporated with four different IPBC contents, PLA grade 2003D was supplied by NatureWorks Asia Pacific Co., Ltd. (Bangkok, Thailand) as a polymeric matrix. CaO of 30 pph was supplied by Thai Poly Chemicals Co., Ltd. (Samut Sakhon, Thailand) as a soil conditioner. IPBC with varying 0, 2.5, 5.0 7.5, and 10.0 ppm was supplied by Troy Asia Co., Ltd. (Bangkok, Thailand) as an antifungal agent. These ingredients were blended and granulated by a twin-screw extruder using a processing temperature of 95–140 °C and rotating speed at 50 rpm. The neat PLA was heated in a 60 °C oven for 8 hr for dehumidity before the blending process. The PLA granules (Both with and without CaO and with/without IPBC) are shown in Table 1.

Table 1 Blending compositions of the PLA, PLA/CaO and PLA/CaO/IPBC granules

Name/formulation	Material compositions		
	PLA (part)	CaO (pph)	IPBC (ppm)
PLA	100	–	–
PLA/IPBC 2.5	100	–	2.5
PLA/IPBC 5	100	–	5.0
PLA/IPBC 7.5	100	–	7.5
PLA/IPBC 10	100	–	10.0
PLA/CaO	100	30	–
PLA/CaO/IPBC 2.5	100	30	2.5
PLA/CaO/IPBC 5	100	30	5.0
PLA/CaO/IPBC 7.5	100	30	7.5
PLA/CaO/IPBC 10	100	30	10.0

pH-value measurement of the PLA and the PLA/CaO granules

The pH value of the PLA and the PLA/CaO granules were investigated by a benchtop pH meter (FiveEasy™ model FE20), supplied by Mettler– Toledo (Thailand) Co., Ltd. An amount of 20 g of the PLA and the PLA/CaO granules in distilled water of 200 ml were stirred and heated by hotplate–magnetic stirrer at 400 rpm and 50 °C for 228 hr. pH values were collected every 24 hr for 10 days (240 h) as a function of the pH collection period, for studying CaO and IPBC releasings for the PLA and the PLA/CaO granules. Buffer solutions were applied for calibrating the pH meter before pH– value collections. The CaO and IPBC releasings were interpreted as pH values of the PLA and the PLA/CaO granules.

Antifungal activities of the PLA and the PLA/CaO granules in the laboratory

P. parasitica was used as fungi in a laboratory gained from the plant pathology research group, Office of Plant Protection Research and Development, Department of Agriculture, Ministry of Agriculture and Cooperatives (Bangkok, Thailand). *P. parasitica* was used for studying the antifungal activities of the PLA and the PLA/CaO granules.

The radius of the inhibition zone or halo test is well–known as the disk diffusion technique which is a qualitative method used for studying antifungal performance for the PLA and the PLA/ CaO granules. Culture media of *P. parasitica* were prepared from a mixture of concentrated carrot juice of 60 ml (Zhang et al., 2020), agar of 3.6 g and distilled water of 300 ml. A mixture was also disinfected by autoclave at 121 °C for 20 min and placed in a sanitized laboratory for cooling before the next step. The mixture for antifungal activity for the PLA and the PLA/CaO granules were poured in equal amounts on Petri dishes using a laminar flow cabinet for controlling non–infection conditions until the carrot juice agar was set. The hyphal tip region of the *P. parasitica* was selected on a dish of carrot juice agar using a cork borer, while a pellet of the PLA and the PLA/ CaO granules were placed nearby. The radius of the inhibition zone of the PLA and the PLA/ CaO granules was determined overnight, as shown in Fig. 1.

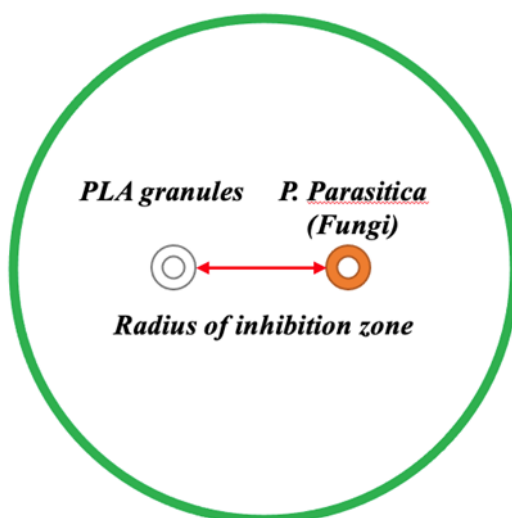


Figure 1 Disk diffusion test of the PLA and the PLA/CaO granules in a Petri dish

The dry weight technique was the quantitative method used for investigating the antifungal performance of the PLA and the PLA/ CaO granules. Culture media for *P. parasitica* for the dry weight method was prepared

the same as in the clear zone method, except for the agar. A cork borer of *P. parasitica* was applied in flasks of carrot juice broth 4 times per flask and 1 g of PLA and PLA/CaO granules were sequentially applied in flasks and incubated for 7 days before suctioning and weighing the fungi. The percentage of viable fungi was calculated using Equation (1).

$$\text{Percentage of Viable fungi (\%)} = \frac{W_c - W_p}{W_c} \times 100 \quad (1)$$

where:

W_c = Weight of fungal growth of control

W_p = Weight of fungal growth of PLA and PLA/CaO granules

Studying the tomato growth using the PLA and the PLA/CaO granules

Seeda tomatoes are an economic crop grown in Thailand. The tested Seeda tomatoes were cultivated at the same time. Seeda tomatoes both at 28–40 days after planting (DAP) and with 40–60 DAP, were used for studying the tomato growth in pots using the PLA and the PLA/CaO granules, as baby and adult tomatoes. Two of the PLA granules both with and without CaO were selected and applied in the uncontrolled soil for tomato growth measurements as a function of granulating periods. The amount of the selected PLA and PLA/CaO granules was 3 g per pot size of 1000 cm² as equivalised using the optimal CaO content (50 kg per rai) (Pulltem et al., 2017; Ruayboonsong et al., 2022).

Disposal and degradation of the PLA and the PLA/CaO granules testing

The exposed soil was ground and sieved with a particle size of 200 μm. A 300 g quantity of the sieved soil and 90 ml of distilled water were mixed for the degradation test, as a mixture of soil. The water was to maintain the soil humidity. A conical flask with a mixture of soil was applied through tubes for moisture and air and other tubes allowed the exit of gases, as shown in Fig. 2. A 3 g quantity of recommended PLA granules was buried in a soil mixture for 45 days, with samples being collected every 15 days for degradation testing. The degradation of the recommended PLA granules was calculated as a percentage weight loss of the neat PLA and PLA/CaO, using Equation 2.

$$\text{Weight loss (\%)} = \frac{W_b - W_a}{W_b} \times 100 \quad (2)$$

where:

W_b = Weight of PLA and PLA/CaO granules before soil burial test

W_a = Weight of PLA and PLA/CaO granules after soil burial test

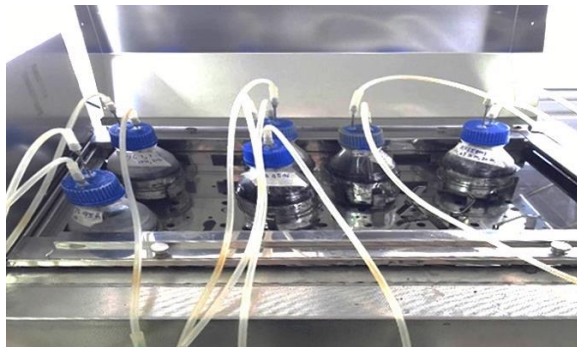


Figure 2 Preparation of soil before degradation test

Results and Discussion

Appearance of PLA and PLA/CaO granules mixed with different IPBC contents

Table 2 shows a photograph of the device in which the PLA and PLA/CaO granules were incorporated with different IPBC contents. It was found that the yellowness of the PLA granules increased with increasing IPBC contents because of the IPBC color. The yellowness of the PLA/CaO granules is not shown due to the opacity and whiteness of the CaO being more influential than the yellowness of the IPBC. The 30 pph CaO content was the most influential of the different IPBC contents. Also, PLA/CaO granules incorporated with different IPBC contents have higher brittleness.

Table 2 Appearance of PLA and PLA/CaO granules with different IPBC contents

Formulation	IPBC content (ppm)				
	0	2.5	5.0	7.5	10.0
PLA					
PLA/CaO					

pH-values of PLA and PLA/CaO granules incorporated with different IPBC contents

Figure 3 shows the pH values of the PLA granules as a function of the pH on each collection day both with and without CaO. Releasing the IPBC of the PLA and the PLA/CaO granules were interpreted as pH values of the PLA and the PLA/CaO granules. It was shown that the acidic condition of the PLA and the PLA/CaO granules increased with increasing the period of pH value collection. An increment of IPBC content from 2.5 ppm to 7.5 ppm showed decreasing pH values. This effectively showed after the pH collection period of 72 hr. A PLA granule incorporated with IPBC of 10 ppm clearly showed the highest acidic condition due to the halide carbamate groups in IPBC being higher and more severe than the lactic acid groups in solution form, as shown in Figure 3(a). In other words, an IPBC content of 10 ppm showed acceptable release. This was shown for PLA granules without CaO contents. For the PLA granules with CaO contents, CaO contents in the PLA/CaO granules compromised the acidic condition (Radziemska et al., 2019). Therefore, IPBC contents did not significantly affect the PLA/CaO granules. This was because the basic condition of CaO reduced the acidity of the PLA/CaO granules, as shown in Figure 3(b).

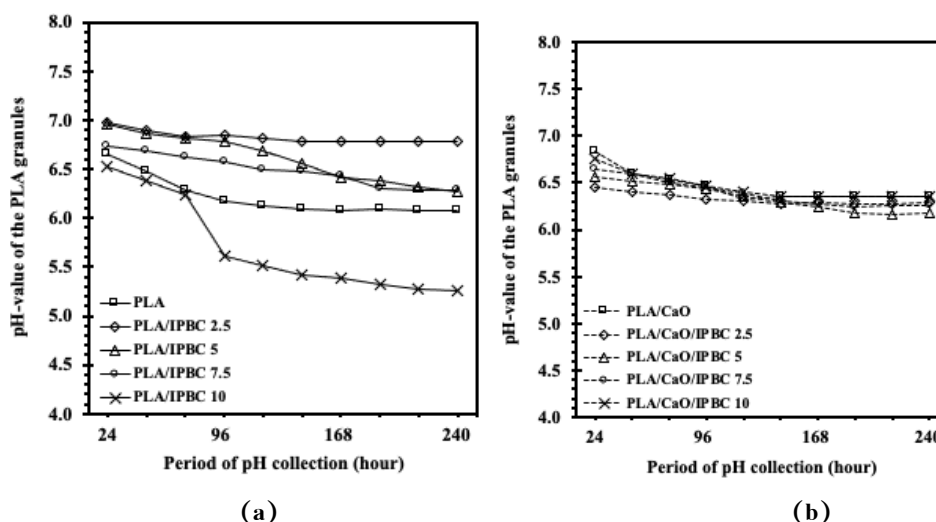












Figure 3 pH-value of the PLA granules as a function of pH collection day(a) without CaO and (b) with CaO

Antifungal properties of the PLA and the PLA/CaO granules adding CaO and IPBC

Disk diffusion method as the qualitative results

Table 3 shows the clear zone of PLA and PLA/ CaO granules with different IPBC contents. It was found that PLA granules incorporated with four different IPBC contents did not inhibit the growth of *P. parasitica*. This was because IPBC contents in the PLA granules did not adequately through the IPBC diffusion. For the PLA/CaO granules, It was found that CaO synergised IPBC inhibited the growth of *P. parasitica*. IPBC Radius of inhibition of the PLA/ CaO granules were 1.4 to 1.9 cm as a function of IPBC contents. This was because the PLA granules mixed with CaO increased the level of IPBC diffusion for the PLA/ CaO granules with different IPBC contents. It seemed that IPBC was promoted and activated by CaO due to CaO is a moisture absorption (Jess et al., 1992; Nakazato et al., 2007) substance to the PLA granules. The moisture is a good intermediary to lead IPBC release out of PLA. However, an isolated CaO in the PLA granule (The PLA/ CaO granule without IPBC content) did not act as an effective antifungal agent due to the CaO in this work was not a CaO nanoparticle (Maringgal et al., 2020).

Table 3 Radius of inhibition zone of PLA and PLA/CaO granules with different IPBC contents

Formulation	Radius of inhibition zone (cm)				
	IPBC content (ppm)				
	0	2.5	5.0	7.5	10.0
PLA	 0.00	 0.00	 0.00	 0.00	 0.00
PLA/CaO	 0.00	 1.40	 1.60	 1.80	 1.90

Dry weight method as the quantitative results

Table 4 shows the dry weight of viable fungi after using PLA and PLA/ CaO granules with different IPBC contents. The quantitative results of the antifungal property after using the PLA and the PLA/ CaO granules similarly tended to the qualitative results. It was shown that the dry weight of *P. parasitica* of the PLA granules increased from 0.081 g to 0.112 g. Conversely, the dry weight of the PLA/ CaO granules decreased from 0.074 g to 0.051g, with increasing IPBC contents of 0 ppm to 10 ppm. This was discussed following the disk diffusion result. A PLA granule with an IPBC content of 7.5 ppm and a PLA/CaO with an IPBC content of 2.5 ppm showed a noteworthy behavior of fungal growth inhibition. This was because of fungicidal- fungistatic behavior (Hawser & Islam, 1999; Kumar et al., 2018). The fungicidal behavior was more influenced than the fungistatic behavior after an IPBC content of 7.5 ppm for a PLA granule. This supported the pH value of PLA and PLA/ CaO granules with different IPBC contents. For a PLA/ CaO granule, the fungicidal region started after an IPBC content of 2.5 ppm was achieved.

Table 4 Dry weight of fungal growth after using the PLA and the PLA/CaO granules











Formulation	Dry weight of fungal growth (g)				
	IPBC content (ppm)				
	0	2.5	5.0	7.5	10.0
PLA	 0.081 ± 0.005	 0.094 ± 0.006	 0.105 ± 0.006	 0.141 ± 0.003	 0.112 ± 0.003
PLA/CaO	 0.074 ± 0.003	 0.081 ± 0.002	 0.065 ± 0.003	 0.062 ± 0.002	 0.051 ± 0.002

Figure 4 shows the percentage of viable fungi for the PLA granules and the PLA/CaO granules with different IPBC contents. It was interpreted that the viable fungi of PLA granules without CaO showed an increase from 95% to 130% and a decrease from 87% to 60% for having CaO as a function of IPBC contents, as shown in Fig. 4. This was discussed the same as disk diffusion and dry weight results.

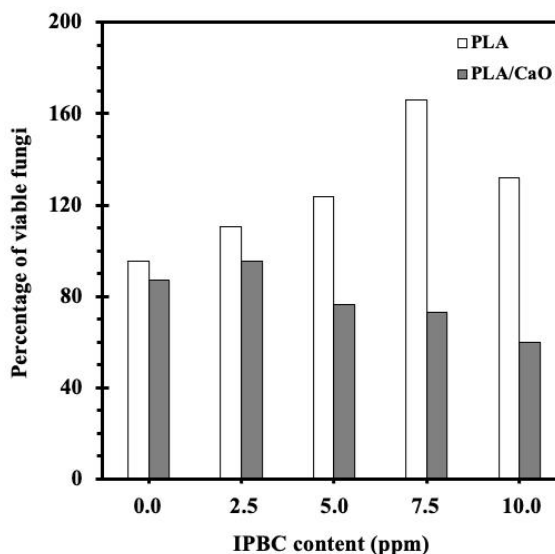


Figure 4 Percentage of viable fungi using the PLA granules with different IPBC contents

Effect of PLA and PLA/CaO granules on Tomato growths

Table 5 shows the effect of selected PLA and PLA/CaO granules incorporated with four different IPBC contents on tomato growths both baby and adult tomatoes. From the sections pH-values and anti-fungal properties, both PLA and PLA/CaO granules with IPBC contents of 2.5 and 10 ppm were selected as representing the developable and optimal conditions, respectively for studying tomato growths. It was shown that the baby tomatoes withered and died with a granulating time of 3 DAP for selective PLA and PLA/CaO granules both with IPBC contents of 2.5 and 10 ppm. However, the selective PLA and PLA/CaO granules retarded the growth of adult tomatoes during the granulating time of 3 to 14 DAP. It can be summarized that the selective PLA and PLA/CaO granules did not work for both baby and adult tomatoes. This was because the plant nutrients from hydrolysis digestion of the selective PLA and PLA/CaO granules were rapidly absorbed by the tomatoes. The tomatoes were not resistant to the acidic-basic condition of plant nutrients of the selective PLA and PLA/CaO granules. However, The PLA/CaO granules with IPBC contents of 2.5 and 10 ppm did not affect tomato growth, but it is appropriate for use as a soil remediator and antifungal agent to prepare the soil before planting.

Table 5 Tomato growths against granulating period using selective PLA granules

Formulation	Baby tomato growth (cm)				Adult tomato growth (cm)				
	Granulating period (Days After Planting; DAP)								
	1	2	3	4	3	6	9	12	14
None (Control)					48	48	> 60	> 60	> 60
PLA/IPBC 2.5	3	3	Withered	Died	45	45	45	45	45
PLA/IPBC 10	3	3	Died	Died	43	44	44	44	44
PLA/CaO/IPBC 2.5	5	5	Died	Died	50	50	50	50	50
PLA/CaO/IPBC 10	3	3	Died	Died	39	39	39	39	39

Disposal degradation of the PLA and the PLA/CaO granules

The PLA/ CaO granule with an IPBC content of 10 ppm was selected as a recommended formulation for studying the disposal degradation as a function of the weight of the tested specimens, compared with neat PLA and PLA/CaO granules. It was revealed that after soil burial tests for 15, 30 and 45 days, the weight of a neat PLA granule, a PLA/ CaO granule and a recommended PLA/ CaO granule decreased to 2.93, 2.66 and 2.55 g, 2.49, 2.08 and 1.64 g, and 2.15, 1.91 and 1.58 g, respectively, as shown in Fig. 5. This was because of moisture absorbability of CaO is well-known as a water scavenger (Jess et al., 1992; Nakazato et al., 2007). The mechanism of PLA degradation occurs via a chain scission reaction. At the same time, moisture is a concerned parameter for PLA degradation. In this work, CaO is a moisture-absorbing substance so it attracted moisture into PLA materials

Table 6 shows other disposal degradation results as a percentage of weight loss of PLA granule compared with a neat PLA granule and a PLA/ CaO granule after a soil burial test for 45 days. It was shown that the percentage of weight loss of neat PLA granule, PLA/ CaO granule, and a recommended PLA/ CaO granule increased with increasing soil burial days. Moreover, it was concluded that a PLA/ CaO granule with an IPBC content of 10 ppm had a faster degradation rate than both a PLA/ CaO granule and a neat PLA granule. This was supported by the discussion in Fig. 5. The weight loss percentage of a neat PLA, a PLA/ CaO, and a recommended PLA/ CaO granule after 45 days were 15.0, 45.3 and 47.2%, respectively.

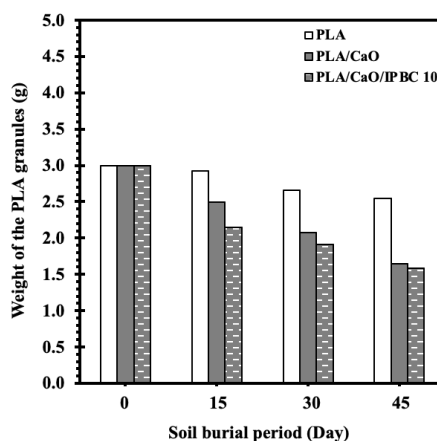


Figure 5 Weight of PLA granule compared with neat PLA and PLA/CaO granules after soil burial test

Table 6 Percentage weight loss of PLA granules compared with neat PLA and PLA/CaO granules after soil burial test

Formulation	Percentage weight loss of PLA granules (%)			
	Soil burial days			
	0	15	30	45
PLA	0.0	2.5	11.3	15.0
PLA/CaO	0.0	16.9	30.7	45.3
PLA/CaO/IPBC 10	0.0	28.4	36.4	47.2

Conclusion and Suggestions

This work presented a potential use of polylactic acid added with IPBC and CaO for Seeda tomato growth as represented for agricultural applications and use as antifungal agents to prepare the soil before planting and compostable soil remediator. The findings of the work can be summarized as follows:

- An acidic condition of the PLA granules increased with increasing IPBC contents from 0– 10 ppm but compromised by CaO. An IPBC was an effective antifungal agent.
- IPBC was promoted and activated by CaO. Growth of *P. parasitica* can be inhibited by CaO synergised IPBC for the PLA/CaO granules.
- The PLA/ CaO granules with the addition of IPBC contents were an effective antifungal agent. A PLA/CaO granule with an IPBC content of 2.5 ppm can be developed to compete with an unused condition.
- A PLA/ CaO granule with an IPBC content of 10 ppm was recommended for agricultural applications. Moreover, a PLA/ CaO/ IPBC 10 acted as an effective antifungal agent and a soil bioremediator for tomato growth.
- Maximum percentage of weight loss of a PLA/ CaO granule with an IPBC content of 10 ppm was influenced by moisture and initial PLA loading.
- The experimental results suggested that a PLA/CaO granule with an IPBC content of 10 ppm (47.2%) had a faster degradation rate than a PLA/CaO granule (45.3%) and PLA granule (15.0%), respectively.

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Author Contributions

Jariyaporn Boonchanamontree: contributed to conceptualization, design of methodology, collection of data, performed the analysis and manuscript review

Napawan Ocharos: contributed to conceptualization, design of methodology, collection of data, performed the analysis and manuscript review

Ekachai Wimolmala: contributed to data analysis and interpretation, manuscript review and critical revision of important intellectual content

Kulnida Taptim: contributed to conceptualization, design of methodology, providing of materials subjects, data analysis, and interpretation, manuscript writing and critical revision of important intellectual content

Conflict of Interests

All authors declare that they have no conflicts of interest.

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