



Original Article

Allelopathic hormesis and slow release of lantana (*Lantana camara* L.) callus extract

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ABSTRACT

Lantana camara L. is believed to contain efficient allelochemicals which can affect many plant species. Using allelopathic chemicals to reduce agrochemical doses could benefit humans and the environment. This research aimed to induce lantana callus, to determine its hormesis and to provide a slow-release formulation of callus extract using calcium alginate beads. The results showed that callus was induced and proliferated on Murashige and Skoog (MS) medium containing 21.5 μ M 1-naphthalene acetic acid (NAA) in combination with 22.5 μ M N⁶-benzyladenine (BA) and MS medium containing 0.5–2.5 μ M 2,4-dichlorophenoxyacetic acid (2,4-D). MS medium containing NAA and BA produced light green and white compact callus (callus NB) while MS medium containing 2,4-D produced light brown compact callus (callus D). Callus D had an inferior effect on seed germination and seedling growth of *Brassica campestris* var. *chinensis* to that of callus NB which totally inhibited seed germination at 0.8% extract. The total fresh weight of seedlings increased when treated with aqueous extract of callus D. An extract concentration of 0.8% or 1.0% induced high total fresh weights of 314.7% and 264.6% of the control, respectively. A slow-release formulation of callus D extract in alginate beads was proven by reduction of the allelopathic effect. Beads with 0.0–5.0% extract had no effect on the germination rate and beads with 1.0–4.0% extract did not reduce the total fresh weight of seedlings. This research suggested hormesis of lantana callus and a slow-release formulation using encapsulation as a novel technique for the further development of natural herbicides.

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Introduction

It has been recognized that some secondary metabolites are produced in plants as defense systems under stress conditions (An, 2005). These substances called allelochemicals have been known for over 2000 years in many plant species such as *Oryza sativa* L. (Rimando et al., 2001), *Triticum aestivum* L. (Wu et al., 2001) and *Lantana camara* (Hussain et al., 2011; Tadele, 2014; Veraplakorn, 2017). Allelopathic chemicals have been reported as effective plant growth regulators, herbicides, insecticides and antimicrobial crop protection products (Cheng and Cheng, 2015). Allelopathic effects comprising a biphasic dose response defined as hormesis, stimulation at low dose and inhibition at high dose, can affect a plant's vicinity including other plants and microorganisms (An, 2005).

Hormesis is a phenomenon that has been known for decades in the toxicological field including in herbicides and phytotoxins (Duke et al., 2006; Mattson, 2008; Liu et al., 2011). The effects of a hormetic

herbicide have been reported for many phenomena such as increased plant growth, induced sucrose accumulation and promotion of pest resistance (Dalley and Richard, 2010; Belz et al., 2011). Hormetic herbicide should be an interesting concept, since low dose application can save cost, reduce pollution and reduce the number of resistant weeds (Belz et al., 2011; Bhadoria, 2011). Until now, there has been no known report applying allelochemicals commercially since the dose usage has proven to be difficult and the mechanism of hormesis in each plant is still unknown (Cedergreen et al., 2007; Belz et al., 2011; Vargas-Hernandez et al., 2017).

It has long been known that using synthetic herbicides continuously in heavy doses could damage the environment and also induces herbicide-resistant weeds (Khanh et al., 2007; Bhadoria, 2011). More recently, many researches have conducted studies to minimize the dependency on synthetic herbicides by trying natural allelochemical and hormetic herbicides (Bhadoria, 2011; Belz et al., 2011; Vargas-Hernandez et al., 2017). Slow-release systems to control the supply of agrochemicals have been reported that can minimize environmental impact because of the reduction in the degradation process, such as photolytic degradation, hydrolytic

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degradation and microbial degradation. Alginate beads are another slow-release formulation which has been reported to control weeds in the long term and has been recommended to improve water holding capacity and reduce soil compaction and erosion (Campos et al., 2015).

Lantana camara L. is a flowering ornamental plant belonging to the family Verbenaceae (Reddy, 2013; Mishra, 2015). Allochemicals have been identified in the whole-plant extract of lantana including monoterpenes and sesquiterpenes, flavonoids, iridoid glycoside, furanonaphoquinones, STH steroids, triterpenes and diterpenes (Mishra, 2015). The allelopathic effect of lantana has been reported on many plant species such as *Brassica juncea* L., (Ahmed et al., 2007), *Oryza sativa* L. (Hossain and Alam, 2010), and *Phaseolus radiatus* (Gantayet et al., 2014). In addition, the extract of lantana from callus and *in vitro* leaves has been found to inhibit the seed germination and seedling growth of many species such as *Salvinia molesta* (Saxena et al., 2013), *Brassica campestris*, *Ipomoea aquatica*, *Sorghum bicolor* L. and *Zea mays* (Verapalakorn, 2017).

This research was conducted to induce lantana callus and to investigate the hormetic effect of the aqueous callus extract. Slow release of lantana allelochemical was examined using encapsulation within a calcium alginate bead.

Materials and methods

Callus induction

Lantana callus was induced from the leaf of *in vitro* plantlets cultured on Murashige and Skoog (MS) medium. Each leaf from the first node was cut across the midrib twice and cultured on seven media formulae: MS medium without plant growth regulator (hormone-free medium), MS medium containing 21.5 μ M naphthalene acetic acid (NAA) in combination with 22.5 μ M N6-benzyladenine (BA) as well as MS medium containing 0.5 μ M, 1.0 μ M, 1.5 μ M, 2.0 μ M and 2.5 μ M 2,4-dichlorophenoxyacetic acid (2,4-D). The relative growth rate ($RGR = 100 \times \text{dry weight of treatment} / \text{dry weight of control}$) of callus was recorded after 2 wk. Initiated callus was separated from the leaf explant and proliferated on each of the same media formulae for use in the next experiment.

Callus proliferation

Callus was proliferated by cutting it into small pieces of (50 mg each) and culturing on six media formulae: MS medium containing 21.5 μ M NAA combination with 22.5 μ M BA and MS medium containing 0.5 μ M, 1.0 μ M, 1.5 μ M, 2.0 μ M and 2.5 μ M 2,4-D. The callus dry weight was recorded after 2 wk.

Allelopathic effect of aqueous callus extract

Callus extract preparation

Two types of lantana callus were induced on MS medium containing 21.5 μ M NAA in combination with 22.5 μ M BA (callus NB) or only 0.5 μ M 2,4-D (callus D). Callus was proliferated by subculturing to fresh medium of the same composition every 4 wk for 4 passages before extraction. Callus was dried at 60 °C for 24 h and subsequently powdered using an electric blender. The aqueous extract was prepared by measuring powder to 0.2 g, 0.4 g, 0.6 g, 0.8 g and 1.0 g and soaking in 100 mL distilled water. The aqueous extracts were kept at 5 °C for 24 h. The extract solution was subsequently filtered through Whatman No.1 filter paper.

Allelopathic effect of aqueous callus extract on seed germination

Whatman No.1 filter paper was placed in sterile 12 cm Petri dishes. The extracts of callus D and callus NB of each concentration

were added to separate Petri dishes (9–10 mL) which was an appropriate amount to keep the seeds moist enough for germination and growth. The control treatment was treated only with distilled water. Twenty seeds of *Brassica campestris* var. *chinensis* were placed in each Petri dish with five replicates at each concentration and incubated at room temperature for 7 d. The percentage germination inhibition (GI) was calculated using the formula $GI = (C-T)/C \times 100$ where, C is the germination percentage of the control and T is the germination percentage of the treatment.

Allelopathic effect of aqueous callus extract on seedling growth

Seedlings of *B. campestris* aged 7 d were grown in plastic pots containing perlite with five seedlings per pot. Each concentration (0.0–1.0%) of callus D extract solution was watered onto each pot (10 mL per pot) once a week. There were three replicates in each treatment. After 4 wk, the results were collected.

Allelopathic effect of encapsulated callus extract

Preparation of encapsulated callus extract with calcium alginate bead

For alginate bead preparation, the 100 mL callus extract of each concentration (0.0–1.0%) was mixed with 2.0 g of sodium alginate and subsequently dropped in a 50 mM CaCl_2 solution to form alginate beads. Perlite was mixed well with the alginate beads of callus extract for use in the growing media in the experiments.

Allelopathic effect of encapsulated callus extract on seedling growth

Seedlings of *B. campestris* aged 7 d were grown in plastic pots each containing 0.5 g of perlite mixed with callus extract alginate beads. Five seedlings were grown in each pot. There were three replicates in each treatment. The fresh weight, dry weight, shoot length and root length were collected after 4 wk.

Allelopathic effect of encapsulated callus extract on seed germination

Seeds of *B. campestris* were germinated on seed trays containing perlite mixed with callus extract alginate bead (five seeds per hole). There were eight replicates in each treatment. The fresh weight, dry weight, shoot length and root length were collected after 4 wk.

Statistical analysis

The analysis of variance (ANOVA) was performed to determine the effect of callus extract on seed germination and seedling growth. Where treatment effect was significant, Tukey's B multiple range test was applied. Differences were considered significant at $p < 0.05$. All analyses were performed using the PASW Statistics 18 software (SPSS Inc.; Quarry Bay, Hong Kong).

Results

Lantana callus was induced from *in vitro* leaves. For callus initiation, all of the media (with the exception of the hormone-free medium) could produce callus. There were no significant differences among the RGRs of callus derived from the treatments of MS medium added with 21.5 μ M NAA in combination with 22.5 μ M BA or MS medium added with 0.5–2.5 μ M 2,4-D (Table 1). Callus was able to grow well on all of the six media formulae. There were no significant differences among the dry weights of callus proliferated on each treatment (Table 1). Callus induced to form on the different media appeared to be of the individual type. Callus found on the MS medium containing NAA and BA was light green and white and compact (Fig. 1A and B) while callus found on MS medium containing 2,4-D was light brown and compact (Fig. 1C and D).

Table 1

Relative growth rate (RGR) of induced callus and dry weight of callus after proliferation on various media for 2 wk.

Medium	RGR of callus ^a	Dry weight (mg/piece) ^a
Hormone free	100.0 ± 0.0 ^b	–
NAA + BA	176.1 ± 10.3 ^a	1873.1 ± 24.2 ^a
0.5 µM 2,4-D	136.1 ± 7.2 ^{ab}	1875.9 ± 19.3 ^a
1.0 µM 2,4-D	139.9 ± 11.1 ^{ab}	1847.4 ± 16.2 ^a
1.5 µM 2,4-D	162.9 ± 13.5 ^a	1863.5 ± 12.4 ^a
2 µM 2,4-D	157.5 ± 12.8 ^a	1883.7 ± 18.1 ^a
2.5 µM 2,4-D	162.8 ± 11.4 ^a	1834.2 ± 24.7 ^a

NAA = 1-naphthalene acetic acid; BA = N6-benzyladenine; 2,4-D = 2,4-dichlorophenoxyacetic acid.

^a Mean values (±SE) with different ≤ superscript letters within each column denote significant ($p < 0.05$) differences between groups.

Allelopathic effect of callus aqueous extract on seed germination

Seed of *B. campestris* was germinated and treated with the extract of callus NB and callus D to determine their allelopathic efficiency. Callus NB had a stronger allelopathic effect on seed germination than callus D. The 50% GI value of callus NB (0.48%) was lower than that of callus D (0.76%). No seedlings germinated in callus NB extract which was higher than 0.6%. On the other hand, seed was still able to germinate in callus D extract at 0.8% and 1.0% where the germination percentage were 35.2% and 42.0%, respectively (Table 2). With callus NB, the shoot and root lengths reduced with increased extract concentration. For callus D, the shoot length was not significantly different with 0.0–0.6% concentrations, while the root length significantly decreased at 0.2% and was stable from 0.2 to 0.6%. The extract at 1.0% produced the highest reduction of root length (Table 2).

Allelopathic effect of callus aqueous extract on seedling growth

The aqueous extract of callus D was examined for its allelopathic effect as a treatment on *B. campestris* seedlings. Seedling growth

significantly increased when the extract concentration increased. High total fresh weights were recorded for the extract at concentrations of 0.8% and 1.0% (314.7% of the control and 264.6% of the control, respectively). However, the total fresh weight with 1.0% extract was not significantly different from the total fresh weights with 0.4–0.6% extract (Fig. 2A). The total dry weight with 0.2% extract was higher (145.0% of the control) but not significantly different compared with the control. There was no significant difference in the dry weights with 0.4–1.0% extract (Fig. 2B).

Allelopathic effect of encapsulated callus extract on seed germination

Seed of *B. campestris* was germinated in growing medium mixed with the extract in alginate bead form to estimate the reduction in the allelopathic effect. Increasing the extract concentration did not reduce the germination rate. Seed exposed to the extract beads had a stable germination percentage with 1.0–5.0% extract (90.6–115.6% of the control) which was not significantly different from the control treatment (Fig. 3A). The total fresh weight at 1.0% extract was 63.8% of the control which was significantly lower than that of the control treatment. The extract from 1.0 to 4.0% reduced the total fresh weight range from 55.9 to 63.8% of the control. With 5.0% extract, the total fresh weight (48.0% of the control) was the lowest but was not significantly different from 4.0% extract at 55.0% of the control (Fig. 3B). The total dry weight significantly decreased in the callus extract treatments with the exception of the 3.0% extract. The total dry weight (93.5% of the control) of 3.0% extract increased but was not significantly different from the control treatment. Extract concentrations at 1.0–2.0% and 4.0% had total dry weight in the range 44.5–56.0% of the control and there were no significant differences. The lowest total dry weight was recorded with 5.0% extract at 23.0% of the control (Fig. 3C).

Allelopathic effect of encapsulated callus extract on seedling growth

Slow release of callus D extract was evaluated by growing *B. campestris* seedlings in the growing medium mixed with the extract in alginate bead form. The seedling growth rate was not affected by any concentration of the extract. There were no significant differences among the total fresh weights and dry weights of 0.0–1.0% extracts which were in the ranges 94.1–100.0% of the control and 100.0–107.8% of the control, respectively (Fig. 4A and B).

Discussion

Induction of callus from lantana leaf has been reported using MS medium added with different types of plant growth regulators such as 2,4-D, BA and NAA in combination with BA (Saxena et al., 2013; Veraplakorn, 2016, 2017). In the present research, MS medium containing 21.5 µM NAA in combination with 22.5 µM BA was able to induce lantana callus in similar amounts to the media containing all tested concentrations of 2,4-D. However, the morphology of the callus was different depending on the culture conditions (types and concentration of plant growth regulators) (Veraplakorn et al., 2012; Kumar et al., 2014; Castro et al., 2016). Callus NB which was induced from the medium supplemented with NAA and BA produced light green and white compact callus. Callus D was light brown and compact. These callus types could proliferate on the same media formulae. Callus induced from the same explant on difference media could have various colors and textures (Veraplakorn et al., 2012; Castro et al., 2016). White-greenish and compact callus of Cavalcade (*Centrosema pasuorum* cv. Cavalcade) was reported on MS media containing NAA in combination with BA while friable yellow callus was induced to form on MS medium containing only

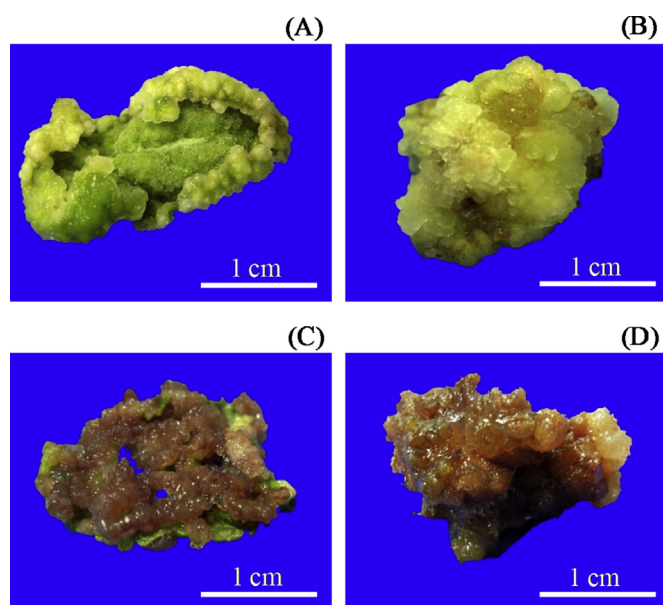


Fig. 1. Callus induction from leaf explant and callus proliferation: (A) callus NB initiation and (B) callus NB proliferation on Murashige and Skoog (MS) medium containing 21.5 µM 1-naphthalene acetic acid combination with 22.5 µM N6-benzyladenine; (C) callus D initiation and (D) callus D proliferation on MS medium containing 0.5 µM 2,4-dichlorophenoxyacetic acid.

Table 2
Effect of callus NB and callus D extract on seed germination of *Brassica campestris* var. *chinensis*.

Extract concentration (%)	Callus NB ^a			Callus D ^a		
	Germination (% control)	Shoot length (% control)	Root length (% control)	Germination (% control)	Shoot length (% control)	Root length (% control)
0.0	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a
0.2	73.5 ± 6.6 ^b	57.0 ± 3.4 ^b	20.7 ± 3.8 ^b	88.6 ± 3.4 ^a	80.0 ± 11.3 ^{ab}	40.0 ± 4.1 ^b
0.4	69.4 ± 2.6 ^b	44.0 ± 2.8 ^c	6.0 ± 1.0 ^c	83.0 ± 5.8 ^a	77.8 ± 7.9 ^{ab}	31.7 ± 3.1 ^b
0.6	44.0 ± 10.9 ^c	39.0 ± 4.2 ^c	2.1 ± 0.2 ^c	79.5 ± 5.7 ^a	75.6 ± 4.2 ^{ab}	35.0 ± 3.1 ^b
0.8	—	—	—	35.2 ± 14.4 ^b	48.9 ± 21.8 ^b	16.7 ± 7.0 ^c
1.0	—	—	—	42.0 ± 5.0 ^b	64.4 ± 4.2 ^{ab}	2.3 ± 0.4 ^d

Callus NB = light green and white compact callus produced using Murashige and Skoog (MS) medium containing 21.5 μ M 1-naphthalene acetic acid in combination with 22.5 μ M N6-benzyladenine; Callus D = light brown compact callus produced using MS medium containing 0.5–2.5 μ M 2,4-dichlorophenoxyacetic acid.

^a Mean values (\pm SE) with different superscript letters within each column denote significant ($p < 0.05$) differences between groups.

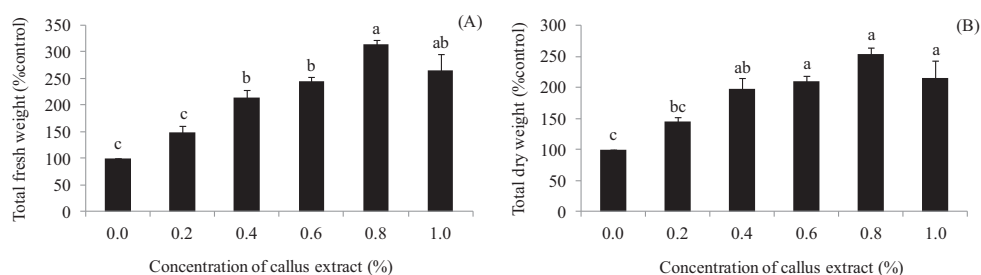


Fig. 2. Seedling growth of *Brassica campestris* var. *chinensis* treated with callus D (see Fig. 1 for definition) aqueous extract for 4 wk: (A) total fresh weight; (B) total dry weight, where columns with different letters denote significant ($p < 0.05$) differences and error bars indicate \pm SE.

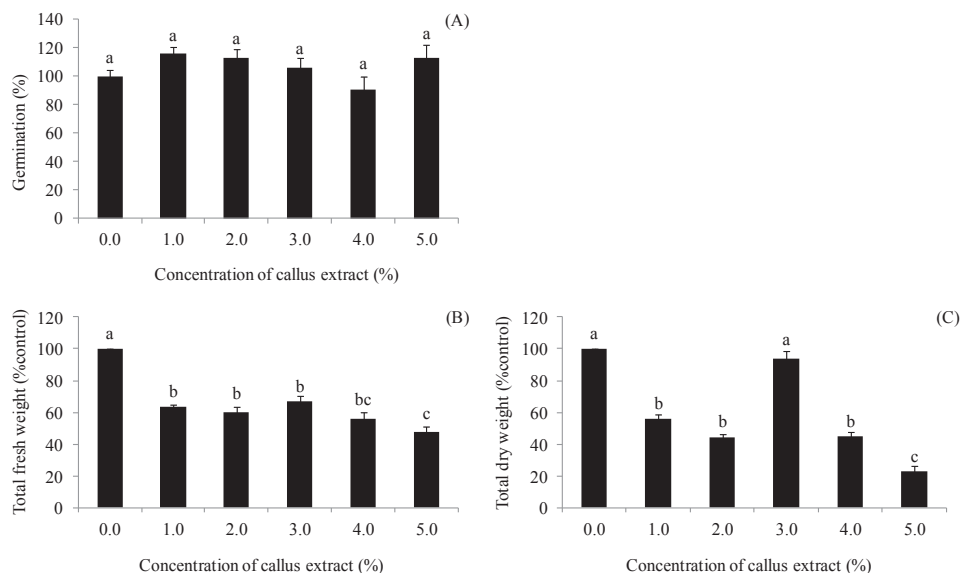


Fig. 3. Seed of *Brassica campestris* var. *chinensis* germinated in perlite mixed with alginate bead of callus D extract (see Fig. 1 for definition) for 4 wk: (A) germination percentage; (B) total fresh weight; (C) total dry weight, where columns with different letters denote significant ($p < 0.05$) differences and error bars indicate \pm SE.

NAA (Veraplakorn et al., 2012). For *Heliotropium indicum*, yellowish or whitish brown and friable callus was produced using 2,4-D while dark brown and compact callus was obtained from the induction of NAA in combination with BA (Kumar et al., 2014).

Interestingly, callus morphology plays a major role in bioactive compound production. Variation in the type and concentration of phenolic compounds produced different callus morphology obtained from different media (Kumar et al., 2014; Castro et al., 2016). In the present study, differences in the allelopathic effects were

found for lantana callus extracted from different type of callus (callus NB and callus D). Both callus NB and callus D exhibited an allelopathic effect, inhibiting the seed germination and seedling growth of *B. campestris*. Interestingly, the allelopathic effect of callus NB was superior to that of callus D. Callus NB was able to completely inhibit seed germination with 0.8% extract while callus D could only inhibit 58% germination with 1.0% extract. This suggested that the variation in the allelochemical efficacy of lantana callus depended on callus morphology.

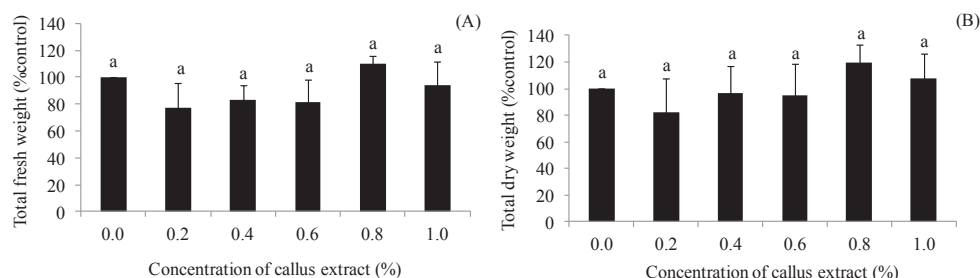


Fig. 4. Seedling growth of *Brassica campestris* var. *chinensis* treated with alginate bead of callus D extract (see Fig. 1 for definition) for 4 wk: (A) total fresh weight; (B) total dry weight, where columns with different letters denote significant ($p < 0.05$) differences and error bars indicate \pm SE.

Callus D produced from 2,4-D would be more convenient and save on cost than the production of callus NB from NAA in combination with BA. In this research, Callus D extract substantially induced seedling growth of *B. campestris* at concentrations from 0.2% to 0.8%. Notably, at the highest concentration of 1.0% extract, the allelopathic effect of lantana tended to decrease as seen by the reduction in the total fresh weight to the same level as with 0.6% extract. This suggested that lantana callus extract possessed hormetic ability. In terms of hormesis, allelochemicals have been indicated to have a negative influence on plants at high concentrations but were able to stimulate growth at low concentrations (Viator et al., 2006; Mattson, 2008; Hadacek et al., 2011; Scognamiglio et al., 2013). Allelopathy in many plant species indicates hormesis (Viator et al., 2006; Geddes et al., 2015). For example, with green sugarcane (interspecific hybrids of *Saccharum* spp.), concentrations of residue extracts above 10% exhibited autotoxicity by delaying early leaf development of sugarcane while a lower concentration of the extract increased bud germination by 45% compared with the control (Viator et al., 2006). Shoot aqueous extract of *Vicia villosa*, *Secale cereal* and *Triticum aestivum* showed hermetic effects by stimulating radicle elongation of *Chenopodium album* L. and *Avena fatua* L. (Geddes et al., 2015). However, there is still a lack of knowledge regarding the application of hormetic allelopathy and research is required on the mechanism and on dose usage (Belz et al., 2011; Vargas-Hernandez et al., 2017). Examining hormesis of callus D aqueous extract which had an inferior allelopathic effect would be an optional method for the further study of allelopathic chemicals as a natural herbicide and plant growth stimulant.

Encapsulation with alginate beads reduces the harmful effect of allelochemicals and provides a long term inhibitory effect (Campos et al., 2015; Huang et al., 2016). This was supported by the present research in which encapsulated callus D extract of 1–5% did not inhibit seed germination but could retard seedling growth. In addition, a concentration lower than 1% of the extract in bead form did not affect seedling growth when treated on seedlings. Interestingly, the dry weight at 3% encapsulated extract was as higher than for the dry weight of the control, perhaps due to the hermetic effect. This may suggest that hormesis of callus D extract was postponed from 0.8% as found when assessing the aqueous extract at 3% because of the encapsulation formulation. Allelochemicals in bead form provide continuous and stable release which is similar to the release of allelochemicals from plant in the natural environment (Huang et al., 2016). The beads are environment-friendly due to their non-toxic and biodegradable natural materials (Abruzzo et al., 2013; Huang et al., 2016). A novel application mode of allelochemicals with long-term inhibitory effects was demonstrated by the continuous-release beads that effectively inhibited cyanobacterial activity in the early bloom phase (Huang et al., 2016). Encapsulated lantana callus extract can be a new method to

produce natural herbicides with slow release for long-term inhibitory effects. If the relation between specific species with regard to their effect on other species can be examined, new natural herbicides which are able to stimulate crop plants will be found.

Conflict of interest

The author declares no conflict of interest.

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