Allelopathic Effect of *Lantana camara in vitro* Tissue – An Interested Natural Herbicide

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ABSTRACT

Allelopathic effect of *Lantana camara* toward many plant species has been reported. Plant allelochemicals have been indicated efficiently usages of growth regulator, herbicide and insecticide etc. The present research, *in vitro* tissues of lantana, including leaf and two types of callus (NB callus and D callus) was determined allelopathic efficiently. NB callus, a light green and white compact callus, was induced and proliferated on Murashige and Skoog (MS) medium containing 21.5 µM 1-naphthalene acetic acid combination with 22.5 µM N6-benzyladenine. D callus, a light brown compact callus, was induced and proliferated on MS medium supplemented with 0.5 µM 2,4-dichlorophenoxyacetic acid. The extract of leaf, NB callus and D callus at the concentrations of 0-1% were treated on *Sorghum bicolor* seed to determine germination inhibition efficiency. In addition, NB callus extract was treated on seedling of *S. bicolor* and *Brassica campestris* to assess seedling growth inhibition efficiency. The extract of NB callus showed the highest germination inhibition percentage with the lowest lethal dose 50. Low concentration of NB callus extract expressed allelopathic hormesis by increasing seedling growth of *B. campestris* and *S. bicolor*. At 0.2% of the extract concentration induced the highest relative growth rate (RGR) of shoot and root of *B. campestris* for 185.3% and 169.5% of control, respectively. In addition, at 0.4% extract concentration promoted shoot RGR of *S. bicolor* up to 121.1% of control. This suggested an interesting lantana *in vitro* tissue as an allelochemical resource for further study to replace synthetic chemicals for sustainable agricultural development.

Key words: allelopathy, *Brassica campestris*, callus, germination, lantana, *Sorghum bicolor*

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INTRODUCTION

Allelopathic chemicals have been found 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, 2018). Allelochemicals have been reported as an effective plant growth regulators, herbicides, insecticides, and antimicrobial crop protection products (Cheng and Cheng, 2015). It has been known that allelochemicals have biphasic doses responded to other plant species, inhibit seed germination and seedling growth at high concentrations but stimulate at low concentration. This has been defined as allopathic hormesis (Mattson, 2008; Hadacek *et al*., 2011; Scognamiglio *et al*., 2013; Cheng and Cheng, 2015, Veraplakorn, 2018).

Lantana camara (lantana) belongs to the family Verbenaceae. It is a flowering, ornamental, evergreen shrub which well known as a traditional medicine and also as firewood and mulch (Kalita *et al*., 2012). Allelopathy of lantana has been suggested the effect on many plant species such as *Triticum aestivum, Vigna sinensis, Cucurbita pepo* (Hossain and Alam, 2010), *Allium cepa* (Hussain *et al*., 2011), *Lathyrus sativa* (Talukdar, 2013), *Salvinia molesta* (Saxena *et al*., 2013), *Phaseolus radiatus* (Gantayet *et al*., 2014), *Brassica campestris*, *Ipomoea aquatic*, *Sorghum bicolor* and *Zea mays* (Veraplakorn, 2017). Allelopathic hormesis of lantana callus has also been reported (Veraplakorn, 2018). In addition, many research suggested allelopathic efficiency of the lantana tissue extract as fungicidal, insecticidal, herbicidal and nematicidal properties (Kalita *et al*., 2012; Reddy, 2013).

It has been well known that plant *in vitro* tissue is capable of inducing similar bioactive compounds as found from natural plants (Hussain *et al*., 2012). Callus and cell suspension culture is a novel technique for secondary metabolite production because of advantages for large scale and continuous culture. In addition, callus produces a superior amount of secondary metabolite in many plant species compare with whole plants from *ex vitro* condition (Tan *et al*., 2010; Vijaya *et al*., 2010; Hussain *et al*., 2012). Callus tissue, however, initiated from the same explant cultured on different media could be different in color and texture (Veraplakorn *et al*., 2012; Castro *et al*., 2016). Different types of callus could produce a variety of active ingredients (Castro *et al*., 2016). In addition, different types of lantana callus showed variously effect of allelochemical on *B. campestris* (Veraplakorn, 2018).

To develop a sustainable agriculture plan, allelochemical is an interesting product which can efficiently be applied as growth regulators, herbicides, insecticides, and antimicrobial crop protection products (Cheng and Cheng, 2015). This research was aimed to investigate the allelopathic potential of lantana *in vitro* tissue for further study to replace synthetic chemical usages.

MATERIALS AND METHODS

Preparation of plant material

Single shoots of lantana were surface sterilized by soaking in 1.0% NaOCl for 15 min and subsequent rinsing three times in sterile distilled water for 10 min. Shoots were initially cultured on Murashige and Skoog (MS) medium. Aseptic shoots were multiplied by culturing on MS medium supplemented with 4.5 µM N6-benzyladenine (BA) for 8 wk. Each single shoot was subcultured to MS medium without plant growth regulator for plant material preparation.

Lantana callus was induced from *in vitro* leaf cultured on MS medium (Veraplakorn, 2016). Each of leaf from the first node was cut across the midrib twice and cultured on two media formulae to induce a light green and white compact callus (NB callus) and a light brown compact callus (D callus): First, the MS medium supplemented with 21.5 µM NAA and 22.5 µM BA for NB callus and second, the MS medium supplemented with 0.5 µM 2,4-D for D callus. Both types of callus were proliferated by cutting into small pieces and subculturing onto the same media formulae every 4 weeks.

Preparation of leaf and callus extracts

Lantana leaf was harvested from *in vitro* shoots cultured on MS medium without plant growth regulator. Two types of callus (NB callus and D callus) and leaf were dried at 60°C for 24 hr and subsequently powdered using an electric blender. The powder was measured to 0.2, 0.4, 0.6, 0.8 or 1.0 g and prepared aqueous extracts by soaking in 100 mL distilled water at 5°C for 24 hr. The extract solution was subsequently filtered through Whatman No.1 filter paper.

Allelopathic effect of leaf and callus extracts on seed germination

Petri dishes of 12 cm in size were placed with Whatman No.1 filter. The extracts of leaf, NB callus and D callus of each concentration (0, 0.2, 0.4, 0.6, 0.8 and 1.0% w/v) were added to each Petri dish for 10 mL to keep the seeds almost soak. The control treatment was treated only with distilled water. Twenty seeds of *Sorghum bicolor* were placed in the Petri dishes with 5 replicates at each concentration and incubated at room temperature for 14 days. The germination percentage and the length of the primary root and the main shoot were determined. The results were reported in term of the germination inhibition percentage (IP) and the relative growth rate (RGR). The IP=(C–T)/ C×100, where C is the germination percentage of the control and T is the germination percentage of treatment. The RGR=100×shoot or root length of the treatment / shoot or root length of the control.

Allelopathic effect of NB callus extract on seedling growth

Seedlings of *Brassica campestris* and *S. bicolor* were grown in small test tubes added with 10 mL of NB callus extract. The control was treated with distilled water. All treatments were incubated under natural light and temperature condition. The survival percentage and the seedling dry weight

were determined after 14 days. It was reported in terms of the relative growth rate (RGR=100×dry weight of the treatment / dry weight of the control).

Statistically analysis

The experiment was conducted in Completely Randomized Design (CRD) which includes six treatments. Each treatment was replicated at five times with 20 seeds per replication. Equal variances were tested using Levene's method. Where significant differences were found due to treatment, Tukey's B multiple range test was applied. Differences were considered significant at $p \leq 0.05$. All analyses were performed using the PASW Statistics 18 software (SPSS Inc.; Quarry Bay, Hong Kong).

RESULTS AND DISCUSSION

Allelopathic effect of leaf and callus extract on seed germination

Lantana *in vitro* tissue; leaf and two types of callus were determined their allelopathic effect. NB callus and D callus extract revealed germination inhibition effect but leaf extract did not show this efficiency. According to the germination inhibition, NB callus and D callus showed lethal dose 50 (LD_{50}) of the extract concentrations at 0.5 and 2.8%, respectively (Figure 1). Leaf extract, however, from 0.2-1.0% were found only 1% IP consequently, it could not showed LD_{50} in this research. This contradicted to the previous work which leaf extract was superior over callus (Veraplakorn, 2017). It may assume that, the present work, the extract volume nearly soaked the whole seeds and a longer time period ensured to destroy new emerged shoots under callus treatment. *In vitro* tissues contained allelopathic effect similar to whole plants from the natural environment that relevant to the suggestion of Hussain *et al*. (2012). In addition, different kinds of callus can produce various active ingredients and express variable allelopathic efficiency (Veraplakorn *et al*., 2012; Castro *et al*., 2016; Veraplakorn, 2018).

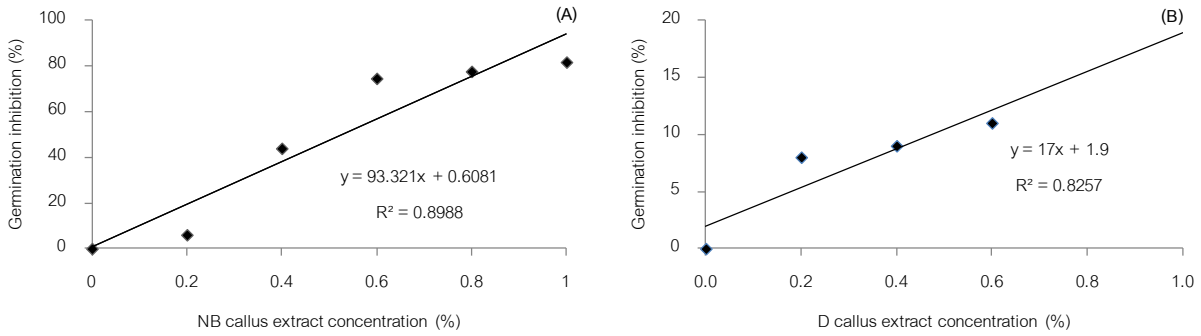

Figure 1 Allelopathic effect of germination inhibition on *Sorghum bicolor* seed: (A) NB callus (B) D callus.

Increasing of the extract concentration reduced seed germination and the RGR of seedling of *S. bicolor*. This agreed with the effect of the lantana tissue extract on many plant species (Hossain *et*

al., 2010; Saxena *et al*., 2013; Gantayet *et al*., 2014; Veraplakorn, 2018). In addition, the lower concentration of all type extracts could induce more significantly inhibition of the root RGR than that of the shoot RGR (Table 1). Normal seedlings could not develop since their roots were reduced radicle elongation and destroyed to become necrotic tissue (Ahmed *et al*., 2007). The root was the first part exposing to allelochemical suggesting that root appeared to be more sensitive than shoot. NB Callus extract, at the low concentration of 0.4% could significantly reduce the RGR of shoot and root (Table1). This indicated the higher effect on seed germination and seedling growth inhibition found in the NB callus extract when compared with other tissues. According to the distinguish effect of NB callus, it was conducted the next experiment to study the allelopathic hormesis effect on seedling growth of *B. campestris* and *S. bicolor*.

Table 1 Allelopathic effect of *in vitro* tissue extract of *Lantana camara* on *Sorghum bicolor* seed germination for 7 days.

Extract concentration (% w/v)	Leaf		NB Callus		D Callus	
	Relative growth rate		Relative growth rate		Relative growth rate	
	Shoot	Root	Shoot	Root	Shoot	Root
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	98.0±2.1^{ab}	74.7±2.2^{b}	32.8±3.4^{b}	22.4±1.5^{b}	35.0±3.0^{b}	69.6±10.4^{b}
0.4	94.4±1.9^{ab}	72.0±4.7^{bc}	12.4±1.7^{c}	9.2±0.2^{c}	35.6±2.3^{b}	66.7±4.8^{b}
0.6	92.8±2.8^{ab}	68.2±2.4^{bc}	8.7±0.7^{c}	4.2±0.6^{d}	32.2±4.2^{b}	40.3±5.7^{c}
0.8	90.5±1.9^{c}	61.9±4.2^{bc}	7.8±0.4^{c}	3.4±0.2^{d}	31.1±1.8^{b}	33.3±7.5^{c}
1.0	90.5±1.9^{c}	60.6±3.2^{c}	6.6±0.1^{c}	2.3±0.2^{d}	36.7±2.5^{b}	30.4±4.2^{c}

* mean values (±SE) with different lowercase, superscript letters within each column denote significant ($p \leq 0.05$) differences between groups.

NB callus showed the higher the allelopathic effect on *B. campestris* than that of the effect on *S. bicolor*. The extract concentration at 0.8% did totally inhibit seedling growth of *B. campestris*. For *S. bicolor*, however, at the highest concentration of the extract was found survival seedling for 40.0% (Table2). Lantana tissues showed a variety of allelopathic effects on the germination and seedling growth of each species (Ahmed *et al*., 2007; Hussain *et al*., 2011; Tadele, 2014, Varaporn, 2017). Normally, an increase of the extract concentration could reduce the survival rate and the RGR of seedlings. NB Callus, however, at low concentration of the extract showed an allelopathic hormesis effect with increasing of the RGR of both *B. campestris* and *S. bicolor*. Notably, at 0.2% of the extract concentration, the RGR of shoot and root of *S. bicolor* was increased up to 185.3 and 169.5% of the control. The extract concentration at 0.4% could induce the RGR of *S. bicolor* shoots up to 121.1% of

the control (Table 2). Allelopathic hormesis of lantana tissue to stimulate other plants has been reported such as *Zea mays* and *Eleusine coracana* (Tadele, 2014) and *B. campestris* (Veraplakorn, 2018). In general, seedling growth was more sensitive than seed germination (Chon and Nelson, 2010). The present research, however, seedling was more tolerant with 40.0% survival percentage at 1.0% extract.

Table 2 Allelopathic effect of NB callus on seedling growth of *Brassica campestris* and *Sorghum bicolor* after growing for 14 days.

Extract concentration (% w/v)	*Brassica campestris**			*Sorghum bicolor**		
	Survival (%)	Relative growth rate		Survival (%)	Relative growth rate	
		Shoot	Root		Shoot	Root
0.0	100.0	100.0±0.0^{b}	100.0±0.0^{b}	100.0	100.0±0.0^{ab}	100.0±0.0^{a}
0.2	100.0	185.3±14.6^{a}	169.5±2.7^{a}	100.0	100.0±5.7^{ab}	71.8±8.7^{b}
0.4	80.0	47.1±7.9^{c}	12.3±2.3^{c}	100.0	121.1±9.3^{a}	55.7±4.7^{bc}
0.6	10.0	21.8±5.6^{c}	10.0±1.1^{c}	90.0	100.0±5.7^{ab}	41.8±3.4^{cd}
0.8	0.0	-	-	80.0	76.0±7.4^{b}	28.4±4.1^{de}
1.0	0.0	-	-	40.0	74.8±6.7^{b}	20.4±3.6^{e}

* mean values (±SE) with different lowercase, superscript letters within each column denote significant ($p \leq 0.05$) differences between groups.

CONCLUSION

Lantana *in vitro* tissue contains allelopathic efficacy. NB Callus has the highest allelopathic efficiency on germination inhibition and seedling growth of *S. bicolor*. Allelochemical of NB callus did higher affect on seed at the germination stage than that of seedling stage. Different plant species variously responded to allelochemical as found the higher tolerant toward callus NB extract of *S. bicolor* when compared with *B. campestris*. In addition, at low concentration of the NB callus extract showed an allelopathic hormesis effect by inducing the higher RGR of *S. bicolor* and *B. campestris*. This suggested that applying allelochemical as weed control with appropriate concentration and timing of each plantation is necessary to be concerned.

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