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Allelopathic Potential of Essential Oil from Bottle Brush (*Callistemon lanceolatus* DC.) on The Germination and Growth of *Echinochloa crus-gall* L.

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A study was conducted to determine the allelopathic effects of essential oil from bottle brush (*Callisremon lanceolatus* DC.) on seed germination and seedling growth of *Echinochloa crus*-galli (L.) Beauv. The essential oil at concentrations of 10, 15, 20 and 25 µl/plate were loaded on a piece of filter paper attached to the inner side of the cover of the Petri-dish. The distilled water was used as the control. The results showed that the essential oil from bottle brush at concentration of 25 µl/plate had hightly inhibition effect on seed germination and seedling growth of E. crus-gall in continuous experiment, natural product herbicide from C. lanceolatus. Essential oil in emusifiable concentrates formulation (50% active ingredient; ECC). The EEC at concentration of 0.1-0.8 µl/ml were bioassayed on seed germination of *E. crus-galli*. The results showed that ECC at 0.8 µl/ml inhibited seed germination by 36.11%. Additionally, the effect ECC on seed imbibition and α-amylase activities of *E. crus-galli* seed were studied. The result showed that seed imbibition and α-amylase activities of *E. crus-galli* seed increased by prolonging time whereas decresed with increasing concentrations of ECC.

Keywords: Essential oil, *C. lanceolatus*, *E. crus-galli*, α-amylase, Imbibition, Germination

Introduction

The control of weeds can be regarded as critical issue for farmers. The interference of weeds in agricultural areas reduces the quality of agricultural products and causes huge economic loss to farms. Thus, the use of synthetic herbicides has become a very common practice for the control of weeds are being despite its widespread use for the control of weeds, there is extensive discussion of the damage they cause to both man and the environment. (Chou *et al.*, 2010). Thereby, developing naturally occurring agrochemicals to replace the synthetic agrochemicals becomes an interesting subject in sustainable

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agriculture There have been some studies about the effects of plant synthesis for the use of herbicide. allelopathy was discovered by Molish and meant that a “plant releases compounds to either stimulate or inhibit the growth of other plant growing in the same habitat in natural and agricultural ecosystem” (Molish, 1937) The use of allelochemicals involved in allelopathic interactions could satisfy the requirements for weeds management and crops protection. Alves *et al*. (2014) reported that the essential oils of *Cinnamomum zeylanicum* Ness, *Lippia sidoides* Cham. and *Cymbopogom nardus* L. the volatile extracts of plants inhibited seed germination and root growth of seedlings of *Bidens pilosa* L., which shows allelopathic potential and the concentration of 0.08% of oils caused the overall deterioration of the roots and death of seedlings of *B. pilosa*. Rahimi *et al*. (2013) noted that Allelopathic effect of some essential oils on seed germination of *Lathyrus annuus* and *Vicia villosa* it can release poisonous effect on the *L. annuus* and *V. villosa* it can be soon that essential oil of plant have a tendency to replace the synthetic agrochemicals.

The bottle brush (*Callistemon lanceolatus* DC.) is a genus of 34 species of shrubs belonging to family Myrtaceae found wildly only on the Australian continent but now all species are endemic to Australia. The genus is known in folk medicine for its anticough, antibronchitis, and insecticidal effects and its volatile oils has been used as antimicrobial and antifungal agents. Moreover antiphylococcal, nematicidal, larvicidal, pupicidal, antithrombotic activities and antioxidant activities (Sumitra and Shiva, 2014). Rassaeifar *et al*. (2013) noted that allelopathic effect of essential oil extracted from leaves of *Eucalyptus globulus* which is the plant in family Myrtaceae it can release poisonous effect on the *Amaranthus blitoides* and *Cynodon dactylon* it can be soon that essential oil of plant have a tendency to replace the synthetic agrochemicals

The objectives of this study to be investigate the effects of essential oil from bottle brush (*Callistemon lanceolatus* DC.) on seed germination of *Echinochloa crus-galli* (L.) Beauv. This paper may be of benefit for development as bioherbicides in future.

Materials and methods

Plant material

The study was carried out at King Mongkut’s Institute of Technology Ladkrabang, Bangkok, Thailand. The essential oil from the leaves of *C. lanceolatus* was extracted by distillation method with water at 95 to 100 ℃ for 2 hours. The experiments were conducted in laboratory. Seeds of *E. crus-galli* were collected from paddy fields at Phitsanulok, Thailand, placed in the shade at room temperature for 3 months, and then incubated in a hot-air oven at 45 ℃ for 48 h to break their dormancy (Poonpaiboonpipat *et al*., 2013).

Inhibitory effect of the essential oil from C. lanceolatus on germination and seedling growth of E. galli seeds

This study was conducted under laboratory conditions using Petridish test. The essential oil at concentration of 10, 15, 20 and 25 µl were loaded on a piece of filter paper attached to the inner side of the cover of the Petri-dish. Distilled water was used as the control. Petri dishes (9-cm diameter) were lined with two layers of germination paper moistened with 5 mL of distilled water and 20 seeds of E. crus-galli were placed. To test the inhibitory effect of C. lanceolatus essential oil, different amounts of oil Petri dishes was added on a piece of filter paper attached inside of the cover Petri-dish placed in a growth chamber at 25-32 ℃, 12 h/12 h dark/light, and relative humidity of ~80%. The rate of germination was measured every day and shoot and root lengths of the seedlings were measured 7 days after treatment. The percentage of germination inhibition were calculated according to the following equation: (Chung *et al.*, 2001)

Inhibition (% of control) = [1 - (sample extracts/control) ×100]

Inhibitory effect of the natural product from C. lanceolatus on germination and seedling growth of E. galli seeds

The essential oil in emulsifiable concentrates formulation (50% active ingredient; ECC) was prepared by mixing with tween80 and DMF (dimethylformide). The treatments of ECC at concentrations of 0.1, 0.2, 0.4 and 0.8 µl/ml, distilled water was used as the control. Petri dishes were lined with two layers of germination paper moistened with 5 ml of each concentration tested and 20 seeds of E. crus-galli were placed per Petri dish. Four replicates were maintained per treatment in a completely randomized manner in a growth chamber with a temperature of 25–32 ℃, 12h/12h dark/light, and relative humidity of ~80%. After 7 days the number of seeds that germinated were counted, and their roots and shoots lengths were measured. The inhibitory or stimulatory percent was calculated using the following equation given by Chung *et al*. (2001).

Inhibitory effect of the natural product from C. lanceolatus on seed imbibition and α-amylase activity of E. galli seeds

Measurement of seed imbibitions was performed using method of Turk and Tawaha (2003) with slight modifications. Four replicates of 30 healthy seeds *of E. crus-galli* were weighed and recorded as original seed weight (W_1). These seeds will be separately germinated in ECC and distilled water as control (according to above treatment). Seed weights were recorded as final seed weight (W_2) for each treatment and exposure time. Water uptake (seed imbibition) percentage was calculated from following equation:

$$\text{Water uptake (\%)} = [(W_2 - W_1) / W_1] \times 100$$

Measurement of activity of α-amylase was made according to the method of Bernfeld (1955) and Sadasivam (1996). After measuring water uptake, seeds (30 seeds of *E. crus-galli* for one determination) were homogenized with 4 ml ice-cold solution of 0.1M $CalCl_2$ and centrifuged at 10,000 rpm for 20 minutes at 4 ºC. Supernatant was used as enzyme extract. The α-amylase activity was assayed by measuring rate of generation of reducing sugar from soluble starch. The reaction medium (3 mL) contained 1 mL of 1% soluble starch in acetate buffter solution at pH 5.5 and 1 mL of enzyme. The assay medium was incubated for 15 minutes at 37 ºC. The reaction was terminated by addition of 1 mL DNS reagent (40 mM 3.5-dinitrosalicylic acid, 0.4 N NaOH and 1M K-Na tartrate), and immediately heated in a boiling water bath for 5 minutes. The mixture was cooled under running tap water. The intensity of color was measured as absorption at 560 nm in a spectrophotometer. The experiment was replicated four times in a completely randomized design (CRD). A standard graph was prepared using maltose, and the amount of α-amylase present in sample were calculated from standard curve and expressed as μmol maltose/min/g

Statistical analysis

The experimental design was completely randomized with four repetitions. The data were subjected to analysis of variance and when the effects of treatments showed significant differences ($P < 0.05$), using Tukey's test.

Results and Discussion

Inhibitory effect of the essential oil from C. lanceolatus on germination and seedling growth of E. galli seeds

The inhibitory effects of essential oil form *C. lanceolatus* on the germination and seedling growth of E. crus-galli shows in Figure 1. The results indicated that the germination and growth of *E. crus-galli* decreased significantly under the effect of essential oil. At concentration of 15 µl, the germination of *E. crus-galli* was inhibited by 28.35%. By increasing the dose of application at 20 and 25 µl, the inhibition magnitude was increased to 41.79 and 68.65%, respectively. In general, the inhibitory effect was more on root length than on shoot length. At the highest concentration of 25 µl, root length was completely inhibited, whereas shoot length decreased by 71.05%. Vishwakarmaa and Mittal (2014) also reported that *Eucalyptus tereticornis* have high allelopathic activity, essential oil from leaves with was inhibited seed germination and seedling development of *E. crus-galli* seed.

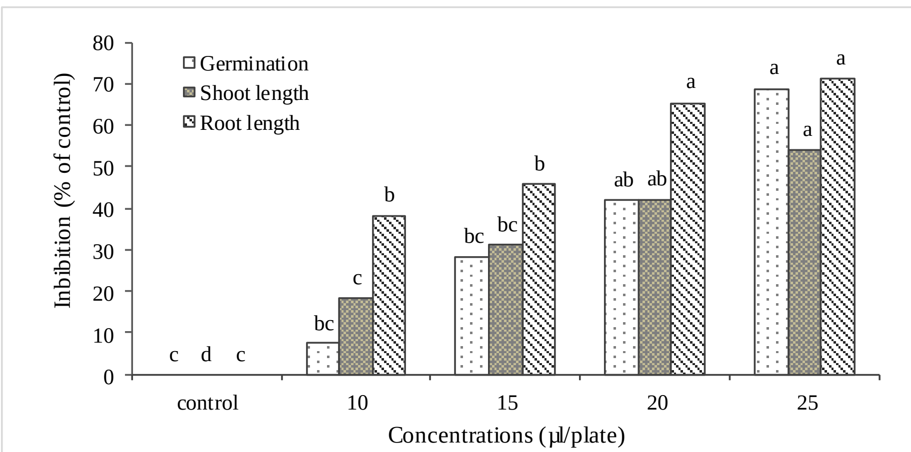

Figure 1. Effects of essential oil from *C. lanceolatus*. on seed germination and seedling growth of *E. crus-galli* seeds. The values represent the means. Different letters indicate significance differences ($p<0.05$) between treatments.

Inhibitory effect of the natural product from C. lanceolatus on germination and seedling growth of E. galli seeds

The effects of natural product from C. lanceolatus (ECC) on germination and seedling growth oif *E. crus-galli* were showed in Figure 2. The germination of *E. crus-galli* was significantly reduced. In general, a dose-response relationship was observed and the emergence decreased with increasing in concentration of ECC. At the highest concentration of 0.8 µl/ml of ECC showed highly inhibition effect on shoot (40.06%) and root length (44.66%), respectively. However, at the lowest concentration of 0.1 µl/ml, there were promotive effects on shoot and root length. These results indicate that ECC contains some inhibitors of the principle of inhibiting germination and seedling growth. The results obtained in the present study are parallel to earlier reports documenting the growth inhibitory activity of aromatic plants, including *Eucalyptus* species which is in the same currency as *C. lanceolatus* and their volatile oils. For instance, volatile oil (0.12-0.30 mg/ml) from *Eucalyptus citriodora* inhibit seedling growth and reduced dry weight accumulation in Cassia occidentalis, *Amaranthus viridis* and *E. crus-galli* by ≥ 50% (Batish *et al.*, 2004). Also, it was demonstrated that the volatile oil from *Eucalyptus citriodora* inhibits the germination and seedling growth of ragweed Parthenium hysterophorus. Thus, these could be used for weeds management (Kohli *et al.*, 1998).

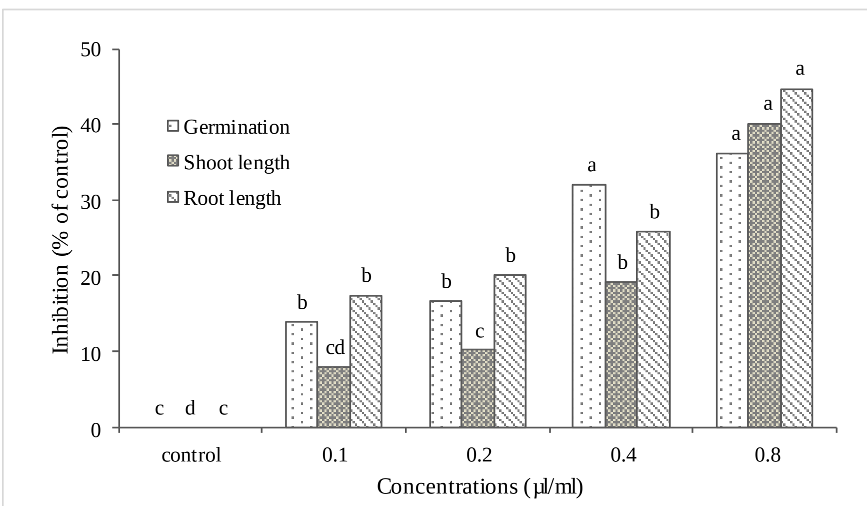

Figure 2 Effects of natural product from essential oil of *C. lanceolatus* on seed germination and seedling growth of E. crus-galli seeds. The values represent the means. Different letters indicate significance differences ($p<0.05$) between treatments.

Inhibitory effect of the natural product from C. lanceolatus on seed imbibition and α-amylase activity of E. galli seeds

The inhibition effect of natural herbicide product from *C. lanceolatus* on seed imbibition and α-amylase activity of *E. crus-galli* seeds were shown in Figure 3. The percentage of imbibition exhibited a marked increase by prolonging the imbibition periods. The time of imbibtion in the control seeds were about 12, 24 and 36 hr, where 17.33, 25.94 and 28.07% respectively. For all treatment concentrations, no significant differences in imbibition after the 12, 24 and 36 hr imbibiton time. This is different from previous studies reported that effect essential oil from cassia (*Cinnamomum cassia*) on seed imbibition of *E. crus-galli* exhibited seed increased by prolonging time whereas decreased with increasing concentrations of essential oil (Ittiwechchai *et al*., 2014).

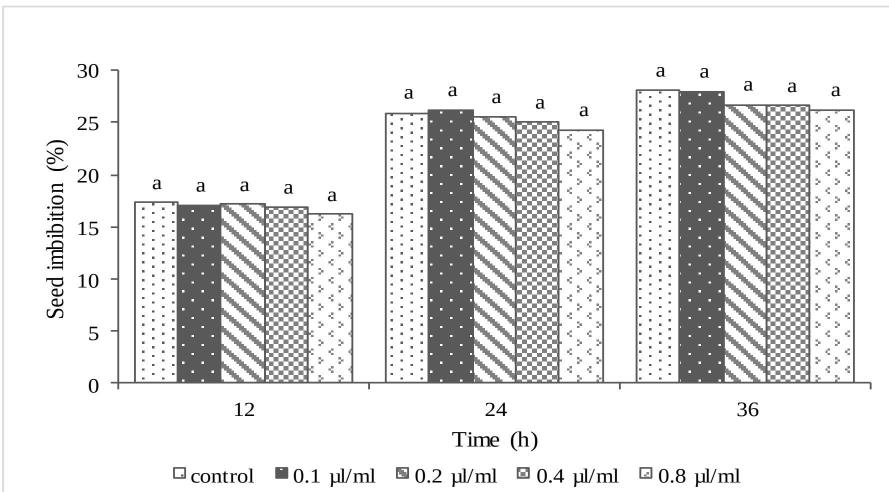

Figure 3 Effects of natural herbicide product from *C. lanceolatus* on seeds imbibition of *E. crus-galli* seeds at different imbibition periods. The values represent the means. Different letters indicate significance differences ($p<0.05$) between treatments.

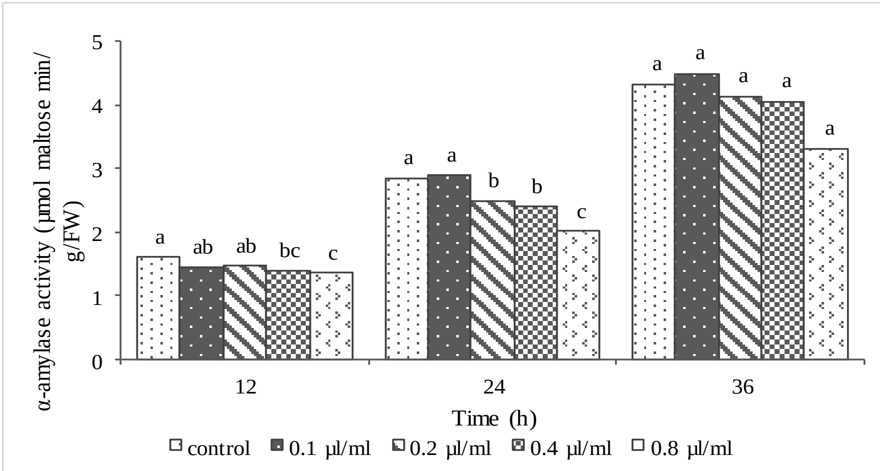

Figure 4. Effects of natural product herbicide from *C. lanceolatus* on α-amylase activity of *E. crus-galli* seeds at different imbibition periods. The values represent the means. Different letters indicate significance differences ($p<0.05$) between treatments.

Figure 4 shows the activities of α-amylase in *E. crus-galli* seeds treated with various concentrations of ECC. The results showed that α-amylase activities increased by prolonging time whereas decreased with increasing concentrations of ECC. The activities of α-amylase were significantly inhibited at all concentrations tested for a soaking period of 12 and 24 h. However, the activity of α-amylase was no significantly inhibited at all concentrations for a soaking period of 36 h. This is different results from previous studies. Poonpaiboonpipat *et al.* (2013) noted that effects of essential oil from *Cymbopogon citratus* inhibit α-amylase activities. At 36 h, the inhibitions of activities in seeds treated with oil were 20.55-57.45% at concentrations of 1-8 µl/Petri-dish.

Conclusion

The results showed that the essential oil from *C. lanceolatus* had high inhibition effect on seed germination and seedling growth of *E. crus-galli* seeds. The inhibitory effect of natural product of essential oil from *C. lanceolatus* in emulsifiable concentrates formulation (50% active ingredient; ECC) was higher than pure essential oil. The inhibition on seed germination of *E. crus-galli* caused by inhibited seed imbibition and the activity of α-amylase in *E. crus-galli* seeds.

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