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Original Article

# Allelopathic effect of *Lantana camara* and *Chromolaena odorata* leaf extracts on plant germination

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## Abstract

Allelopathy is a biological process where plants affect, often inhibitory, the growth and germination of other species within their space. This study aimed to demonstrate and compare the allelopathic effect of *Lantana camara* and *Chromolaena odorata* leaf extracts on plant germination, with *Vigna radiata* as the test plant. Leaf extracts were assayed at 10%, 25%, 50%, 75%, and 100% concentrations, and the corresponding allelopathic effects were compared to that of control. Findings indicated that *L. camara* and *C. odorata* leaf extracts inhibited *V. radiata* seedling growth and germination in increasing concentrations, with *C. odorata* leaf extract exhibiting greater inhibitory effect. The estimated marginal mean lengths (in cm) of root, hypocotyl, and epicotyl of *V. radiata* are 0.917, 5.937, and 3.263 under the control; 0.195, 0.813, and 0.499 under *L. camara*; and 0.101, 0.217, and 0.051 under *C. odorata*, respectively. Phytochemical analysis showed presence of several allelochemicals in both leaf extracts. These compounds were suspected to be the primary drivers of the observed allelopathic effect. It is suggested that the quantitative phytochemical analysis and herbicidal properties of *L. camara* and *C. odorata* be studied further.

**Keywords**: *Lantana camara*, *Chromolaena odorata*, Allelopathy, Invasive Alien Species, Bioassay

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## Introduction

Allelopathy is a biological process by which plants influence the growth of their neighbors through releasing allelochemicals (Sodaeizadeh and Hosseini, 2012). At low allelochemical concentrations, plants commonly exhibit stimulatory allelopathic effect on its neighbors, whereas high concentrations can suppress the growth and germination of nearby plants (El-Kenany and El-Darier, 2013). Allelopathy is primarily exhibited by invasive alien species (IAS). IAS commonly invade accidentally or deliberately places that are not part of their natural habitats, causing imbalance to the ecosystem and depletion in crop production (Callaway and Aschehoug, 2000). However, despite the inhibitory effect of IAS, their allelochemicals were potentially found to be utilized as natural herbicides against other IAS, as an alternative to the commonly used synthetic herbicides that pose soil pollution and health risks to the consumers (Jabran and Farooq, 2013).

Two of the most common IAS in the Philippines are *Lantana camara* and *Chromolaena odorata*. *L. camara* is a perennial and invasive shrub that can be mostly found in arid areas with high temperature (Mishra, 2015). *L. camara* was observed to exhibit allelopathy, wherein it invades the growth of pastures, thus decreasing plant productivity (Joshi, 2006). Allelochemicals, such as sesquiterpenes, flavonoids, glycosides, and triterpenes, have been found to be present in *L. camara* (Mishra, 2015). *C. odorata*, on the other hand, is a perennial shrub that commonly grow in well-drained soils with low fertility (Mandal and Joshi, 2014). Armed with an exceedingly huge amount of allelochemicals, such as saponins, tannins, terpenoids, alkaloids, flavonoids, and glycosides, *C. odorata* has strong allelopathic effect wherein it invades native plant communities due to its ability to grow rapidly and its high reproducibility of seeds (Hu and Zhang, 2013).

Although natural herbicides seem promising, only limited scientific evidences have been presented to support this claim. Thus, further researches about the potential of IAS as natural herbicide should be conducted. Generally, this study compared the allelopathic effect of *L. camara* and *C. odorata* on plant germination by determining which between the two noxious invasive weeds exhibits greater inhibitory effect. Moreover, this study determined if there is a significant difference on allelopathic effect between control and increasing concentrations as well as to record presence of allelochemicals in the leaf extracts. With these, a firmer findings for more environmental-friendly herbicides against IAS proliferation comes into availability for future researchers.

## Material and Methods

### Plant acquisition

Mature leaves of *L. camara* and *C. odorata*, weighing 500 g each, were acquired from infested areas in Mount Zion, Pangasinan Province, Philippines and placed in polyethylene bags until utilization. Due to easy replication, rapid growth, and observable traits in germination and growth, *Vignata radiata* was chosen as test plant. Seeds of *V. radiata* were obtained from local markets and screened for seed quality. Damaged seeds were then discarded upon visual examination. The plant identification was verified and properly authenticated at University of Santo Tomas Herbarium (USTH).

### Extract preparation

The preparation of the leaf extracts followed that of P et al. (2010). Leaves were sun-dried for 14 days and ground afterwards. The resulting fine particles were dipped into two separate pails for 24 hours, each containing 5 L of methanol, from a basis ratio of 1 g per 10 mL. The produced mixture was filtered by a fluted Whatman No. 1 filter paper (particle retention of 11 µm). The filtrates underwent rotary evaporation for solvent separation at about 70℃, resulting to about 160 mL per leaf extract. Using the extract as stock solution, 10%, 25%, 50%, 75%, and 100% concentrations were prepared by dilution, and their allelopathic effect was tested through seed germination bioassay (Hu and Zhang, 2013).

### Petri dish bioassay

Six set-ups were prepared for the germination bioassay; triplicates for *L. camara* leaf extract (LCLE) and triplicates for *C. odorata* leaf extract (COLE). Each set-up constituted six 9 cm-diameter petri dishes for different concentrations, each of which contained five *V. radiata* seeds with tissue as seedbed. The first petri dish was used for application of distilled water (control), and the succeeding received 10%, 25%, 50%, 75% and 100% extract concentrations. About 5 mL of each concentration was added to each petri dish for the first day. The seeds were kept moist by applying about 1 mL of each extract daily for the next 13 days. After two weeks of observation, germinated seeds were counted based on the presence of radicle (Ahmed et al., 2007). Root, epicotyl, and hypocotyl of seedlings were also measured using a foot ruler.

### Phytochemical analysis

LCLE and COLE were subjected to phytochemical analysis using Molisch, Fehling, Alkaline Reagent, Lead Acetate, Hager, Mayer, Wagner, Ferric Chloride, Borntrager, Keller-Kiliani, Froth, Resin, Liebermann-Buchard, and Salkowski tests to assess the presence of several allelochemicals (Usunomena and Efosa, 2016; Yadav and Agarwala, 2011).

### Data analysis

Germination percentage and marginal mean length were used to determine the magnitude of inhibitory effect on *V. radiata* seedlings. Seedling measurements underwent two-way MANOVA to test the differences between treatments and the control using SPSS version 23. Statistical significance was accepted at $p<0.05$; otherwise, rejected.

## Results and Discussion

Allelopathic effect is exhibited when there is a negative effect in seedling growth and germination, compared to seedlings under the control. Results indicated that there is accumulating evidence on the inhibitory effect of LCLE and COLE on *V. radiata* seedling conditions, seen in the decrease in seedling growth and germination (Abhilasha et al., 2008; Maghrebi and Mirshekari, 2011; Orr et al., 2005). These negative effects showed that the responses of *V. radiata* seedlings are concentration dependent and species specific (Table 1). Findings in this study were found to be consistent with reports of Ahmed et al. (2007), and Hu and Zhang (2013).

### Concentration dependence

Generally, there was a significant difference ($p<0.05$) between the control and increasing concentrations (Table 2). However, there was no significant difference ($p>0.05$) between 50%, 75%, and 100% concentrations with respect to effect on root and hypocotyl length; and between 25%, 50%, 75%, and 100% in epicotyl length. This implies that very few to no seeds germinated under these treatments. Table 1 shows how germination percentage lowers with increasing concentrations. Maximum seed germination, wherein all the seeds germinated, was observed under the control, and 10% LCLE and COLE; and then lowered as the concentration was increased. No seed germination was evident under 50% COLE, and 75% LCLE. Moreover, Figure 1 show differences in marginal mean length of root, hypocotyl, and epicotyl of seedlings under the control, and different treatments. Seedling growth with respect to root, hypocotyl, and epicotyl length is maximum under control, and lowers with increasing concentration. Growth has already been impeded starting from 75% in root, 50% in hypocotyl, and 25% in epicotyl. Due to these negative effects, the relationship between increasing concentration and corresponding inhibitory effect is directly proportional.

**Table 1. Germination percentage of *V. radiata* under different concentrations of LCLE and COLE**

| | Control | LCLE | | | | | COLE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Concentration** | 0 | 10 | 25 | 50 | 75 | 100 | 10 | 25 | 50 | 75 | 100 |
| **No. of Germinated Seeds** | 15* | 15* | 15* | 7* | 0* | 0* | 15* | 10* | 0* | 0* | 0* |
| **Germination Percentage** | **100** | **100** | **100** | **47** | **0** | **0** | **100** | **67** | **0** | **0** | **0** |

*15 total number of seeds were initially present.

**Table 2. MANOVA results (Post Hoc Test – Tukey HSD) of petri dish bioassay of *V. radiata* seedling root, hypocotyl, and epicotyl under different concentrations**

| | | Root | | Hypocotyl | | Epicotyl | |
|---|---|---|---|---|---|---|---|
| **(X) Concentration** | **(Y) Concentration** | **Std. Error** | **Sig.** | **Std. Error** | **Sig.** | **Std. Error** | **Sig.** |
| **0** | 10 | .04287 | .000 | .15503 | .000 | .16214 | .000 |
| | 25 | | | | | | |
| | 50 | | | | | | |
| | 75 | | | | | | |
| | 100 | | | | | | |
| **10** | 25 | .03500 | .000 | .12658 | .000 | .13238 | .000 |
| | 50 | | | | | | |
| | 75 | | | | | | |
| | 100 | | | | | | |
| **25** | 50 | .03500 | .000 | .12658 | .010 | .13238 | 1.000 |
| | 75 | | | | | | |
| | 100 | | | | | | |
| **50** | 75 | .03500 | .766 | .12658 | 1.000 | .13238 | 1.000 |
| | 100 | | | | | | |
| **75** | 100 | .03500 | 1.000 | .12658 | 1.000 | .13238 | 1.000 |

The standard error and significant difference is directed to the corresponding X and Y concentration.

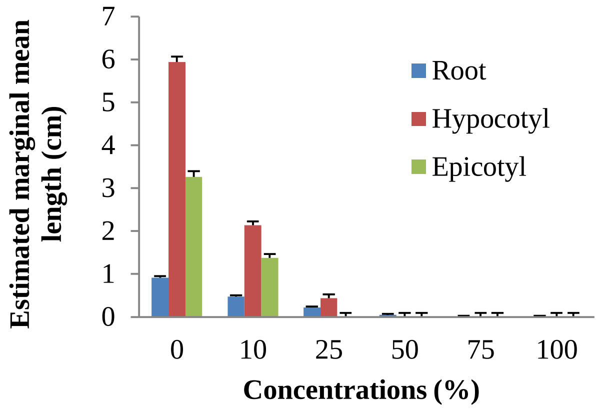


**Figure 1. Estimated marginal mean lengths of *V. radiata* based on concentrations**

**Species specificity**

Table 3 shows that significant differences ($p<0.05$) were present between the effects of control, LCLE, and COLE to *V. radiata* germination and growth. In Table 1, maximum seed germination was evident under the control, 10% LCLE and COLE, and 25% LCLE. Different from 25% LCLE, only ten seedlings germinated under 25% COLE. Moreover, COLE already obstructed seed germination at 50%; while LCLE stopped germination at 75%, with seven germinated seeds still present under 50%.

**Table 3. MANOVA results (Post Hoc Test – Tukey HSD) of petri dish bioassay of *V. radiata* seedlings under different treatments**

| | (X) Treatment | (Y) Treatment | Std. Error | Sig. |
|---|---|---|---|---|
| **Root** | Control | LCLE | .03834 | .000 |
| | | COLE | | |
| | LCLE | COLE | .02214 | .000 |
| **Hypocotyl** | Control | LCLE | .13867 | .000 |
| | | COLE | | |
| | LCLE | COLE | .08006 | .000 |
| **Epicotyl** | Control | LCLE | .14502 | .000 |
| | | COLE | | |
| | LCLE | COLE | .08373 | .000 |

The standard error and significant difference is directed to the corresponding X and Y concentration.

Furthermore, Figure 2 shows that growth of *V. radiata* seedlings is highest in control, lower in LCLE, and lowest at COLE. Due to lower germination and smaller mean lengths, greater inhibitory effect is exhibited by COLE.

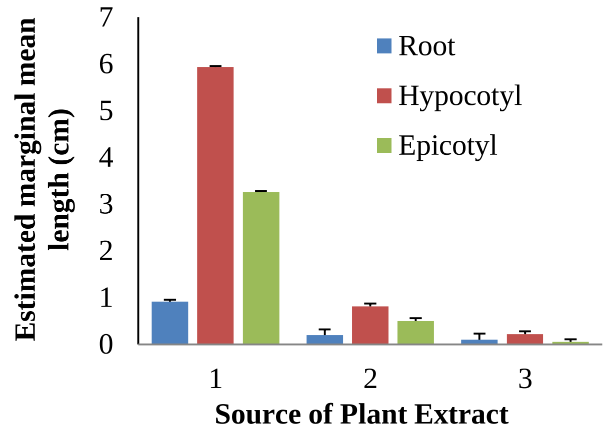


**Figure 2. Estimated marginal mean lengths of *V. radiata* based on extract source**

**Allelopathy**

Allelochemicals drive allelopathy that serves as a plant's protection against herbivory, and way to eliminate competitors (Dewick, 2009). Phytochemical results showed presence of allelochemicals, such as flavonoids, alkaloids, tannins, saponins, and triterpenoids in LCLE and COLE, which caused allelopathic effect exhibited in the experiment (Table 4). However, glycosides were absent in COLE, contrary to Mishra's (2015) finding. This can be attributed to insufficient sensitivity of qualitative tests, and the amount of concentration that was tested by the laboratory, as glycosides can only be found at low concentrations (Chiejina and Onaebi, 2016). These allelochemicals present in LCLE and COLE impose mechanisms on plant growth and germination. Flavonoids disrupt photosynthetic processes by reducing the electron transport rate and inducing membrane depolarization causing the ineffectiveness of the selective permeability of cells (Huang et al., 2015). Alkaloids affect physiological processes in the plants by increasing the temperature of the DNA cleavage, and preventing translation and transcription of DNA (Cheng et al., 2015). Tannins directly affect cellular respiration through inhibiting peroxidase activities, catalase, and cellulose (Li et al., 2010). Saponins cause damage to cell membranes, by disrupting the membrane domain, creating pores, and vesiculation (Augustin et al., 2012) while triterpenoids inhibit seed germination, radicle growth, and the function of photosystem II (Wang et al, 2014).

**Table 4. Phytochemical composition of LCLE and COLE based on presence (+) or absence (-)**

| Metabolites | LCLE | COLE |
|---|---|---|
| Carbohydrates | - | - |
| Reducing Sugars | - | - |
| Flavonoids | + | + |
| Alkaloids | + | + |
| Tannins | + | + |
| Glycosides | - | - |
| Saponins | + | + |
| Resins | - | - |
| Sterols | + | + |
| Triterpenoids | + | + |

Despite finding similar allelochemicals in LCLE and COLE, COLE exhibited greater allelopathic effect due to lower germination percentage and seedling growth. This may be attributed to higher amounts of allelochemical concentration in COLE than LCLE, but quantitative phytochemical analysis must be performed for confirmation. In Rusdy (2015), *C. odorata* was reported to have higher inhibitory effect than *L. camara* on the *Leucaena leucocephala* seedling growth, wherein findings in this research coincides with. However, Rusdy and Ako (2017) revealed that the inhibitory effect observed in the germination and seedling growth of *Centrosema pubescens* was more pronounced with *L. camara* than *C. odorata*. These differences in allelopathic effect indicate that the responses and corresponding effects are concentration dependent and species specific, varying depending on the plant source of allelochemicals and its level of concentration, and to what specific plant receives the effects (Ahmed et al., 2007; Hu and Zhang, 2013)

However, the petri dish bioassay conducted may overestimate the allelopathic effect of allelochemicals compared to actual field situations wherein several extraneous factors are present, such as compounds in soil, water, and temperature (Hierro and Callaway, 2003). Therefore, a combination of laboratory and field experiments are recommended to determine the persistence and bioactivity of allelochemicals *in situ* (Hu and Zhang, 2013; Inderjit & Foy, 2001).

## Conclusion

This study shows that the leaf extracts of *Chromolaena odorata* and *Lantana camara* can suppress the growth and germination of *Vigna radiata* seedlings. The inhibitory effect is directly proportional with the amount of extract concentration with *C. odorata* causing greater inhibition than *L. camara*. Several allelochemicals were found on both plants, confirming their allelopathic ability. Moreover, quantitative analysis, and combination of laboratory and field experiments are recommended to efficiently explore the allelopathic effect of these plants and their potential application as natural herbicides.

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## Contribution of Authors

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Tandoc WC: Designed Research Methodology, Literature Search, Data Collection
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