Plant Omics Journal POJ

POJ 14(01):72-77 (2021) ISSN:1836-3644
doi: 10.21475/POJ.14.01.21.p3396

Assessment of the allelopathic potential of Leucas cephalotes (Roth) Spreng. extracts on the seedling growth of six test plants

Thang Lam LUN[1,2]*, Hisashi KATO-NOGUCHI[1,2]

[1]Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795, Japan
[2]The United Graduate School of Agricultural Sciences, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan

***Corresponding author: tllunpi43@gmail.com**

Abstract

Leucas cephalotes displays a wide range of pharmacological activities and thus has been used as a traditional medicine. However, its allelopathic properties have not yet been studied. In this study, *L. cephalotes* was extracted with aqueous methanol to assess its inhibitory effect and the allelopathic properties. The results showed that the *L. cephalotes* extracts inhibited the shoots and roots growth of cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), barnyard grass (*Echinochloa crus-galli* L.), Italian ryegrass (*Lolium multiflorum* Lam.) and timothy (*Phleum pretense* L.) at concentrations >10 mg dry weight (DW) equivalent extract mL^{-1}. The growth of the test plants decreased with an increase in concentration of the extracts. The concentration needed for 50% inhibition (I_{50} values) of the test plant shoots and roots were in the ranges 4.21 – 49.45 and 4.81 – 21.00 mg DW equivalent extract mL^{-1}, respectively. In all tested plants, the shoots are more sensitive to the extracts of *L. cephalotes* than the roots. The seedling growth of alfalfa displayed the highest inhibition whiles the barnyard grass exhibited the least inhibition. All the test plant species responded to the *L. cephalotes* extracts. The findings of the current study indicate that *L. cephalotes* exhibits allelopathic activity and may possess allelopathic substances.

Keywords: *Leucas cephalotes*; allelopathic properties; medicinal plant; inhibitory effect.

Introduction

Weeds are plants that are more harmful than useful, even after possible beneficial effects have been considered (Pathipati et al., 2011) and diminish the crop productivity by their diverse habits of intrusive with crops growth and culture (Thijs et al., 1994; Kruse et al., 2000). Therefore, herbicides are widely used and play an important role in increasing agricultural production. He et al. (2012) highlighted that herbicide have become the most common used chemical substances all over the world. Since 2007, herbicides have ranked in first place among the three main pesticides of insecticides, fungicides/bactericides and herbicides (Zhang et al., 2011). However, over-use of synthetic herbicides brings about tremendous environmental hazards (Aktar et al., 2009) and has resulted in the growth of herbicide-resistant weeds (Heap, 2018). Excessive use of herbicides induces numerous changes in plant growth such as inhibition of growth, foliar chlorosis, albinism and necrosis (Subba-Rao and Madhulety, 2005). Many herbicides still remain in the environment and cause bio magnifications. Researchers are now seeking out new natural plant products to develop biodegradable and cost-effective herbicides for supportable weed management (Islam et al., 2018). Herbicides from plants are safer and easily biodegradable (Babu et al., 2014). Allelopathy holds the potential for selective biological weed management. The phenomenon of allelopathy refers to chemical interactions between all types of plants. In this process the chemical exudates or leachates released from all parts of a plant can inhibit the growth of a neighbouring one (Scrivanti et al., 2011).

Plant materials possessing phytotoxic potential can be used in agriculture as natural herbicides (Kato-Noguchi et al., 2016). Many reports have documented the profitable use of plants having phytotoxic potential (isolated substances) to suppress weeds in crop fields in place of using synthetic herbicides (Mushtaq et al., 2010). Moreover, plant extracts with phytotoxicity and their phytotoxic substances have no residual toxic effects on the environment (Amb and Ahluwalia, 2016). Accordingly, many investigators pay much attention to potentially finding more allelopathic plants from a variety of plant species (Kuddus et al., 2011). Medicinal plants are a valuable source of secondary metabolites that usually have many biological functions (Hussein and El-Anssary, 2018). Moreover, it has been reported that medicinal plant species possess strong allelopathic potential (Wink, 1999; Qasem, 2002). Therefore, investigators throughout the world are now concentrating on medicinal plants to seek new natural plant products (Sodaeizadeh et al., 2009).

Myanmar, a country with a rich cultural heritage, comprises more than 100 ethnic groups: the Chin people are one major group. The climate is typically temperate with three seasons like other regions of Myanmar. In the rainy season, southwestern monsoon winds bring abundant rainfall to Chin State, creating a habitat that favors the explosive growth of countless plants (Thu et al., 2018). In traditional medicine, *Leucas cephalotes (Roth) Spreng.* has been used as a natural remedy against several afflictions such as jaundice, inflammation, asthma, fever, cough, malaria and anemia (DeFilipps and Krupnick, 2018; Thu et al., 2018). *L. cephalotes*, from the family Laminaceae, is a yearly rainy-season weed. It grows widely in the cultivated fields of Myanmar and other Asian countries such as India and China. The plant is an erect, scabrous or pubescent, stout annual herb, 30 -100 cm high (Kirtikar and Basu, 1999). Its leaves are yellowish-green, the stem is light greenish yellow and the roots are cylindrical, zig-zag, smooth, and long with numerous wiry. Inflorescence is sessile and white, and the fruits are nutlets, smooth and brown (Anonymous, 2001). Biological and pharmacological researchers have shown that, potentially, *L. cephalotes* has many valuable biological properties such as anti-inflammatory (Patel et al., 2015), antioxidant (Rao et al., 2014), antimicrobial (Kumar et al., 2016), antifilarial, antifertility (Bhoria et al., 2013), antiplasmodial, hepatoprotective (Bais and Saiju, 2014), and antidiabetic (Verma et al., 2017). However, there have not been any studies about the allelopathic properties of *L. cephalotes*. This research was conducted to determine if *L. cephalotes* possesses allelopathic substances that could be used to suppress crop weeds for sustainable agricultural production.

Results

The extracts of *L. cephalotes* were assayed to measure the shoot and root growth of each test plant species (cress, lettuce, alfalfa, Italian ryegrass, barnyard grass, and timothy) at various concentrations. The findings indicated that the extracts considerably suppressed the seedling growth of each test plant (Fig. 1 and Supplementary Fig. 2). At 30 mg DW equivalent extract of *L. cephalotes* mL^{-1}, the lettuce seedlings were completely inhibited and the cress, lettuce, alfalfa, Italian ryegrass, barnyard grass, and timothy were significantly inhibited more than 50% to 3.17, 2.70, 1.86, 3.40 and 3.87% of the control shoot length and 3.56, 2.88, 0.90, 2.84 and 3.03% of the control root length. All the tested seedlings except barnyard grass were completely inhibited (100%) at the concentration of 300 mg DW equivalent extract of *L. cephalotes* mL^{-1}, whilst the barnyard grass shoots were inhibited up to 99% and its roots were completely inhibited. Other concentrations also showed different levels of growth inhibition against each species. The correlation coefficients (R) of the concentration of the *L. cephalotes* extracts showed a significant negative correlation with shoot and root length percentage in each test plant (Table 1). The seedlings of each test plant had a highly significant negative correlation with the *L. cephalotes* extracts at ($p < 0.01$).

To suppress 50% of seedling growth (I_{50} values), the concentration of the *L. cephalotes* extracts needed for each species was different (Fig. 2). The most significant difference between the I_{50} values for the shoot and root length was found in barnyard grass followed by cress, Italian ryegrass, and timothy. No significant difference shoot and root length was found in lettuce and alfalfa. The I_{50} values for shoot and root length were within 4.21 - 49.45 and 4.81 – 21.00 mg DW equivalent of *L. cephalotes* extracts mL^{-1}, respectively. Of the test plants, alfalfa was the most sensitive to the *L. cephalotes* extracts, requiring the lowest concentration to inhibit 50% of the growth and barnyard grass was the least sensitive, requiring the highest concentration. The *L. cephalotes* extracts had a stronger effect on the dicotyledons than on the monocotyledons.

Discussion

According to the bioassay results, the *L. cephalotes* extracts inhibited the growth of both the monocotyledonous and dicotyledonous test plants, and the percent of inhibition increased with an increase in extract concentration (Fig. 1 and Supplementary Fig. 2). The extracts from allelopathic plant shows an inhibition of germination and seedling growth of plant bioassay with increasing on a concentration (Poonpaiboonpipat and Jumpathong, 2019). The same concentration-dependent inhibition results have been reported for the medicinal plants *Dischidia imbricata* (Krumsri et al., 2019), *Acacia catechu* (Hossen and Kato-Noguchi, 2020) and *Acacia pennata* (Kyaw and Kato-Noguchi, 2020). These results also correlated with many research findings on inhibitory activity depending on plant extract concentration (Zaman et al., 2018). Sodaeizadeh et al. (2009) also described those differences in the biochemical and physiological nature of test plants may be responsible for the inhibitory effects of the extracts. The concentration-dependent inhibitory pattern of these findings indicates that *L. cephalotes* extracts may possess allelopathic potential. Boonmee et al. (2018) and Kato-Noguchi et al. (2019) reported that a substantial number of phytotoxic substances has been successfully extracted from medicinal plants using aqueous methanol.

For I_{50} values, the barnyard grass requires the highest concentration and alfalfa need the lowest concentration (Fig. 2). The results for barnyard grass are similar to the findings of Krumsri et al., (2019), Hossen and Kato-Noguchi (2020), and Kyaw and Kato-Noguchi (2020), and the results for alfalfa are similar to those of Rob and Kato-Noguchi (2019). These results indicate that growth suppression by the *L. cephalotes* extracts was species dependent. Similar findings on plant extracts have also been reported by Kato-Noguchi et al., (2016) and Boonmee et al., (2018). As shown in Fig. 2, the *L. cephalotes* extracts had more inhibition effect on the dicotyledons than on the monocotyledons. The unequal susceptibility of different species may be due to differences in seed size and seed coat permeability, which influence the absorption of inhibitory substances (Hassan et al., 2012). Kruse et al. (2000) has been indicated that dicotyledons are more sensitive to allelopathic substances than monocotyledons. Additionally, Islam and Kato-Noguchi (2014) described that inhibition of the growth of tested seedlings by extracts of *L. aspera* is mainly due to the allelopathic reaction. In this study, the aqueous methanol extracts of *L. cephalotes* strongly inhibited the seedling growth of the selected test plants. Thus, we suggest that *L. cephalotes* extracts could be used as a natural herbicide with the aim of environmental safety. Further study is needed to identify and isolate the allelopathic substances from *L. cephalotes* extracts.

Table 1. Correlation coefficient between the concentration of the *Leucas cephalotes* extracts and the growth of the six tested plants.

Test plant species	Correlation coefficient (R)	
	Shoot	Root
Cress	- 0.802**	- 0.812**
Lettuce	- 0.820**	- 0.852**
Alfalfa	- 0.592**	- 0.574**
Barnyard grass	- 0.789**	- 0.801**
Italian ryegrass	- 0.768**	- 0.823**
Timothy	- 0.719**	- 0.750**

Asterisks indicate significant level: ** p < 0.01 (two-tailed).

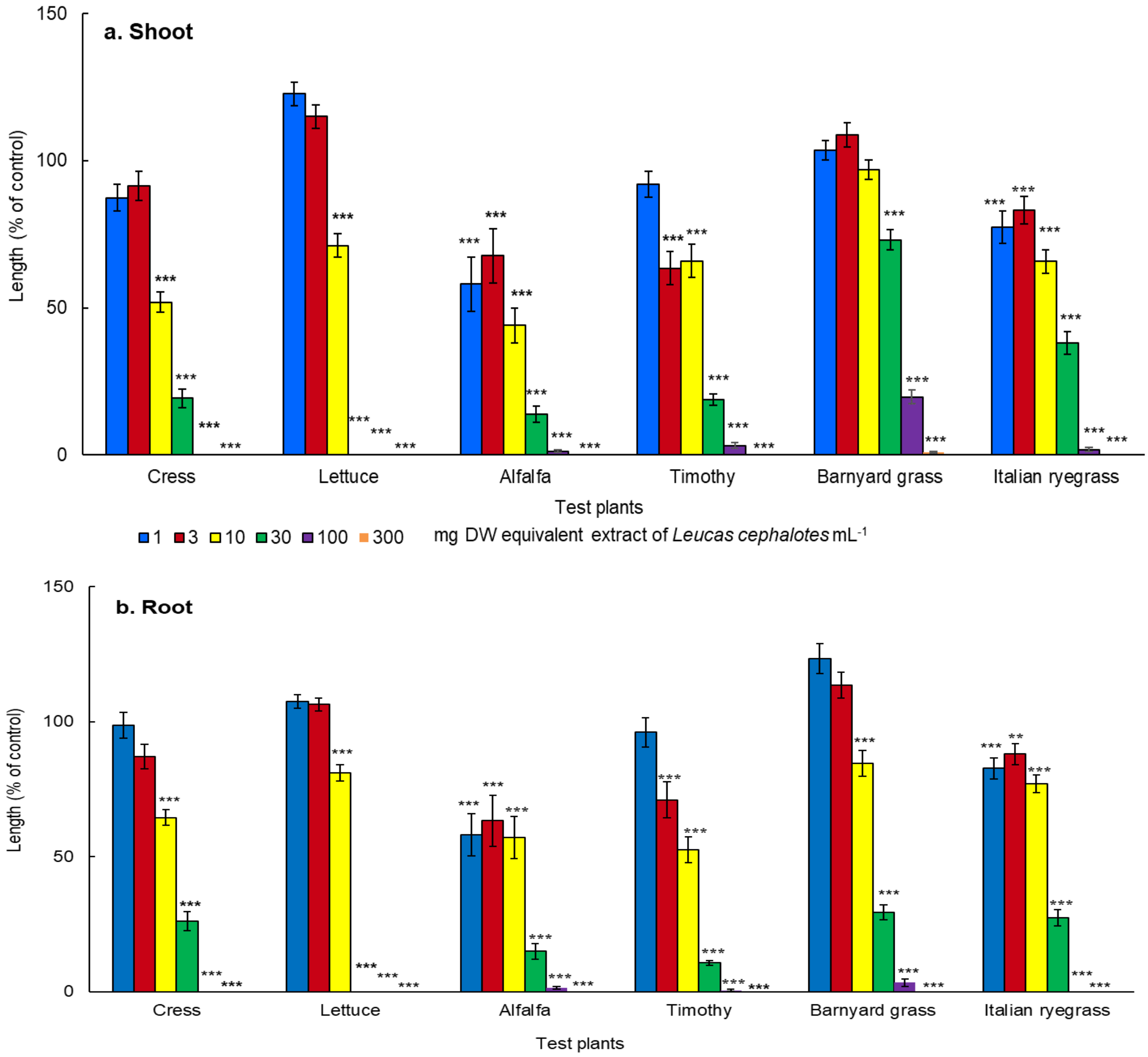

Fig 1. Effect of *Leucas cephalotes* extracts on the (a) shoot (b) root growth of the tested plant species. Seedling growth was checked after two-days of incubation in the dark at 25 °C. Means ± SE from two independent experiments with three replications for each treatment (n=60). Asterisks above each bar indicate significant differences between treatment and control: *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001 (One-way ANOVA, post hoc by Tukey's test).

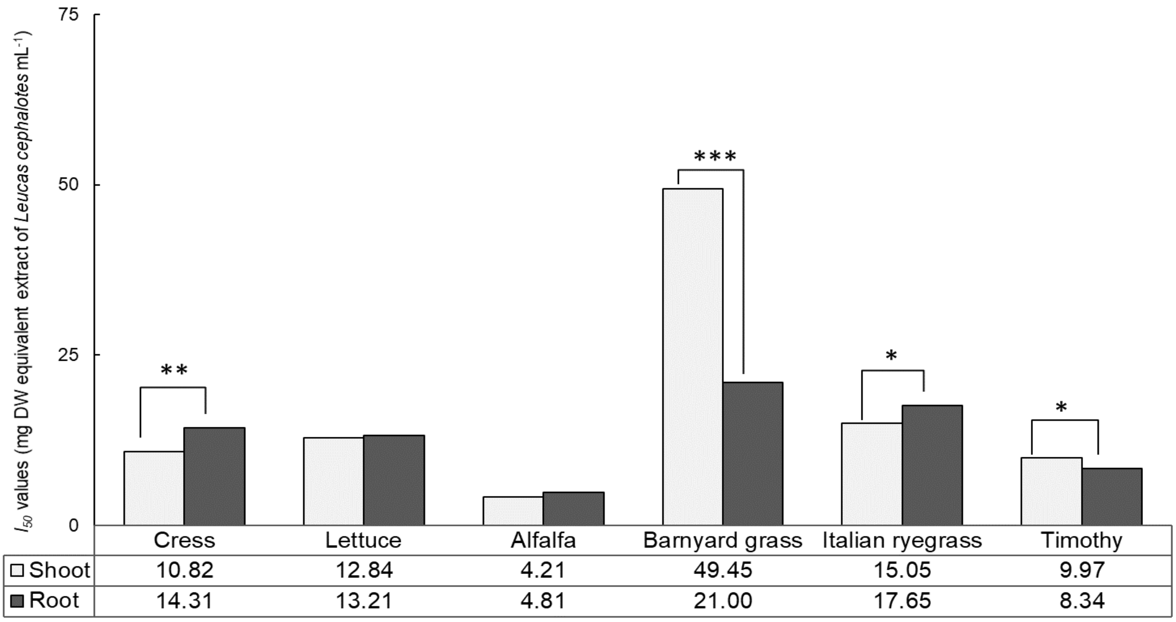

Fig 2. Concentration of *Leucas cephalotes* extracts needed to suppress 50% of seedling growth (I_{50} values) of the tested plants. Asterisks above each bar indicate significant differences between the shoot and root growth of each species: *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001 (paired t-test).

Materials and Methods

Plant materials

Plant materials (*L. cephalotes*) were gathered from the villages near Tiddim Township, Chin State, Myanmar in July-August 2020 (Supplementary Fig. 1). The plant parts of *L. cephalotes* except the roots were washed with tap water to remove mud and other particles, and dried in the shade. The dry samples were then cut into small pieces using scissors, ground into powder, and then stored at 4 °C in a vacuum-sealed plastic package until extraction. For the bioassay, three dicotyledons [cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), and alfalfa (*Medicago sativa* L.)] and three monocotyledons [barnyard grass (*Echinochloa crusgalli* (L.) Beauv.), Italian ryegrass (*Lolium multiflorum* Lam.), and timothy (*Phleum pratense* L.)] were selected. The tested species of monocots were chosen because of their common seedling growth patterns and the dicots because of their distribution as common weeds in the crop fields (Das et al., 2019).

Preparation of plant extracts

The powder of *L. cephalotes* (100 g) was extracted with 600 mL of 70% (v/v) aqueous methanol for 48 h at room temperature in the dark. The extract sample was stirred with a laboratory spatula and filtrated with a vacuum pump and a 125 mm sheet of filter paper (No. 2; Toyo Roshi Ltd., Japan). The residue from the filtration was soaked for 24 h with 600 mL of methanol at room temperature in the dark and filtrated again. Both filtrates were combined and evaporated to complete dryness using a rotary evaporator at 40 °C. The crude extracts were dissolved with 25 mL methanol and kept at 2 °C until the bioassay.

Seedling bioassay and determination of I_{50}

To assess the inhibitory effect of *L. cephalotes*, a bioassay was conducted with six concentrations (1, 3, 10, 30, 100, and 300 mg DW equivalent extract mL^{-1}) and controls. To prepare these concentrations, the *L. cephalotes* was dissolved with methanol and an aliquot of the extracts was added individually to a layer of filter paper (No. 2) in each 28 mm Petri dish. The Petri dishes were dried in a draft chamber to remove all methanol. After that, 0.6 mL of 0.05% (v/v) aqueous Tween 20 solution (polyoxyethylene sorbitan monolaurate; Nacalai, Kyoto, Japan) solution was added to each Petri dish. Then, 10 seeds of cress, lettuce, and alfalfa, and 10 germinated seeds of barnyard grass, Italian ryegrass, and timothy (allowed to germinate for 48 h; incubated in the dark at 25 °C) were placed on the filter paper in the Petri dishes. The Petri dishes with only the seeds and Tween 20 solution on the filter paper were the control. The root and shoot lengths were measured after 48 h incubation in darkness at 25 °C. The percentage of seedling growth was recorded by referring to control seedlings' growth. The inhibition percentage was calculated using the following equation:

$$\text{Inhibition (\%)} = \left[1 - \frac{length\ with\ aqueous\ methanol\ extract}{length\ of\ control}\right] \times 100$$

Statistical analysis

The bioassays were carried out in a completely randomized design (CRD). The treatments and controls were duplicated and replicated three times (10 seeds/replication, n = 60). All experimental data were analyzed using SPSS version 16.0 software. The differences between the I_{50} values of each test plant were analyzed using a paired *t*-test at $p \leq 0.05$. Two-tailed Pearson Correlation was applied to check the correlation between the growth of the tested plants and extract concentration.

Conclusion

The present study investigated the possible allelopathic activity of the medicinal plant against several test plants. The aqueous methanol extracts of *L. cephalotes* suppressed the growth of both monocotyledonous and dicotyledonous species. The growth suppression of the seedlings increased with increasing concentration of the extracts. Thus, the inhibitory activity of *L. cephalotes* extracts suggests that this plant possesses allelopathic potential and may contain allelopathic substances. Further study should be conducted to isolate and characterize any potential allelopathic substances from this plant.

Acknowledgement

We thank the Government of Japan for supporting a scholarship to Thang Lam Lun. We also thank Professor Dennis Murphy, The United Graduate School of Agricultural Sciences, Ehime University, Japan for checking and editing the English of the manuscript.

References

Aktar W, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip. Toxicol. 2:1–12.

Amb MK, Ahluwalia AS (2016) Allelopathy: potential role to achieve new milestones in rice cultivation. Rice Sci. 23:165–183.

Babu GP, Hooda V, Audiseshamma K, Paramageetham C (2014) Allelopathic effects of some weeds on germination and growth of *Vigna mungo* (L). Hepper. Int J Curr Microbiol Appl Sci. 3:122–128.

Bais B, Saiju P (2014) Ameliorative effect of *Leucas cephalotes* extract on isoniazid and rifampicin induced hepatotoxicity. Asian Pac. J. Trop. Biomed. 4:633–638.

Bhoria R, Kainsa S, Chaudhary M (2013) Antifertility activity of chloroform and alcoholic flower extracts of *Leucas cephalotes* (Roth.) Spreng. in albino rats. Int. J. Drug Dev. Res. 5:168–173.

Boonmee S, Iwasaki A, Suenaga K, Kato-Noguchi H (2018) Evaluation of phytotoxic activity of leaf and stem extracts and identification of a phytotoxic substance from *Caesalpinia mimosoides* Lamk. Theor. Exp. Plant Physiol. 30:129–139.

Das IN, Iwasaki A, Suenaga K, Kato-Noguchi H (2019) Evaluation of phytotoxic potential and identification of phytotoxic substances in *Cassia alata* Linn. Leaves. Acta Agric. Scand. B. Soil Plant Sci. 69:479–488.

DeFilipps RA, Krupnick GA (2018) The medicinal plants of Myanmar. PhytoKeys 102:1–341.

Hassan MH, Daffalla HM, Yagoub SO, Osman MG, Gani MEA, Babiker AEGG (2012) Allelopathic effects of some botanical extracts on germination and seedling growth of *Sorghum bicolor* L. J. Agric. Sci. Technol. 3:132–138.

He H, Yu J, Chen G, Li W, He J, Li H (2012) Acute toxicity of butachlor and atrazine to freshwater green alga *Scenedesmus obliquus* and cladoceran *Daphnia carinata*. Ecotoxicol. Environ. Saf. 80:91–96.

Heap I (2018) International survey of herbicide resistant weeds. Retrieved 2020 May 21 from *http://www.weedscience.org*

Hossen K, Kato-Noguchi H (2020) Determination of allelopathic properties *Acacia catechu* (L.f) Willd. Not. Bot. Horti Agrobot. Cluj-Napoca 48:2050-2059.

Hussein RA, El-Assary AA (2018) Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. Herbal Medicine; Builders, P.H., Ed.; IntechOpe.

Islam AKMM, Kato-Noguchi H (2014) Phytotoxic activity of *Ocimum tenuiflorum* extracts on germination and seedling growth of different plant species. Sci. World J. ID: 676242.

Islam AKMM, Yeasmin S, Qasem JRS, Juraimi AS, Anwar MP (2018) Allelopathy of medicinal plants: current status and future prospects in weed management. Sci. Res. J 9:20.

Kato-Noguchi H, Suzuki M, Noguchi K, Ohno O, Suenaga K, Laosinwattana C (2016) A potent phytotoxic substance in *Aglaia odorata* Lour. Chem. Biodivers. 13:549–554.

Kato-Noguchi H, Suwitchayanon P, Boonmee S, Iwasaki A, Suenaga K (2019) Plant growth inhibitory activity of the extracts of *Acmella oleracea* and its growth inhibitory substances. Nat. Prod. Commun. 14:1–5.

Kirtikar KR, Basu BD (1999) Indian medicinal plants, Vol III. Published by National Book Distributor, Dehradun, 2017–18.

Krumsri R, Boonmee S, Kato-Noguchi H (2019) Evaluation of the allelopathic potential of leaf extracts from *Dischidia imbricata* (Blume) Steud. on the seedling growth of six test plants. Not. Bot. Horti Agrobot. Cluj-Napoca 47:1019–1024.

Kruse M, Strandberg M, Strandberg B (2000) Ecological Effects of Allelopathic Plants – a Review. National Environmental Research Institute, Silkeborg, Denmark. 66 pp. – NERI Technical Report No. 315.

Kuddus MR, Ali MB, Rumi F, Aktar F, Rashid MA (2011) Evaluation of polyphenols content and cytotoxic, membrane stabilizing and antimicrobial activities of seed of *Rumex maritimus* Linn. Bangladesh J. Pharmacol. 14:67–71.

Kumar D, Kumar V, Jangra P, Singh S (2016) *Leucas cephalotes* (Spreng): photochemical investigation and antimicrobial activity via cylinderplate method or cup-plate method. Int. J. Pharm. Sci. Res. 1:28–32.

Kyaw EH, Kato-Noguchi H (2020) Allelopathic potential of *Acacia pennata* (L.) Willd. leaf extracts against the seedling growth of six test plants. Not. Bot. Horti Agrobot. Cluj-Napoca 48:1534–1542.

Mushtaq MN, Cheema ZA, Khaliq A, Naveed MR (2010) A 75% reduction in herbicide use through integration with sorghum + sunflower extracts for weed management in wheat. J. Sci. Food Agric. 90:1897–1904.

Patel NK, Khan MS, Bhutani KK (2015) Investigation on *Leucas cephalotes* (Roth.) Spreng. for inhibition of LPS-induced pro-inflammatory mediators in murine macrophages and in rat model. EXCLI J. 14:508–-516.

Pathipati UR, Pala RR, Nagaiah K (2011) Allelopathic effects of *Sterculia foetida* (L.) against four major weeds. Allelopathy J. 28:179-188.

Poonpaiboonpipat T, Jumpathong J (2019) Evaluating herbicidal potential of aqueous–ethanol extracts of local plant species against *Echinochloa crus-galli* and *Raphanus sativus*. Int J Agric Biol 21:648-652.

Qasem JR (2002) Allelopathic effects of selected medicinal plants on *Amaranthus retroflexus* and *Chenopodium murale*. Allelopathy J. 10:105–122.

Rao BB, Kumar SV, Rao BR, Mohan GK (2014) Study of antioxidant activity of different fractions of *Leucas cephalotes* (Roxb.ex Roth) Spreng. World J. Pharm. Res. 3:953–958.

Rob MM, Kato-Noguchi H (2019) Study of the allelopathic activity of *Garcinia pedunculata* Roxb. Plant Omics 12:31-36.

Scrivanti LR, Anton AM, Zydadlo AJ (2011) Allelopathic potential of South American *Bothriochloa* species (Poaceae:Andropogoneae). Allelopathy J. 28:189–200.

Sodaeizadeh H, Rafieiolhossaini M, Havlík J, van Damme P (2009) Allelopathic activity of different plant parts of *Peganum harmala* L. and identification of their growth inhibitors substances. Plant Growth Regul. 59:227–236.

Subba-Rao IV, Madhulety TY (2005) Role of herbicides in improving crop yields. In:Developments in physiology biochemistry and molecular biology of plants, Bose B. and Hemantaranjan A. (Eds.), New India Publishing Agency, New Delhi 1:203–287. ISBN-8189422022.

Thijs, H., Shann, J. R., & Weidenhamer, J. D. (1994). The effect of phytotoxins on competitive outcome in a model system. Ecology, 1959-1964.

Thu ZM, Aye MM, Aung HT, Sein MM, Vidari G (2018) A review of common medicinal plants in chin state, Myanmar. Nat. Prod. Commun. 13:1557–1567.

Verma A, Kumar A, Upreti DK, Pande V, Pal M (2017) Fatty acid profiling and *in vitro* antihyperglycemic effect of *Leucas cephalotes* (Roth) Spreng via carbohydrate hydrolysing enzyme inhibition. Pharmacogn. Mag. 13:22–25.

Wink M (1999) Introduction: biochemistry, role and biotechnology of secondary metabolites. Annual Plant Reviews, 1-16.

Zaman F, Islam S, Kato-Noguchi H (2018) Allelopathic activity of the *Oxalis europea* L. extracts on the growth of eight test plant species. Res. Crop. 19:304-309.

Zhang W, Jiang F, Ou J (2011) Global pesticide consumption and pollution: with China as a focus. Proc. Int. Acad. Ecol. Environ. Sci. 1:125–144.