
**UNIVERSITI PUTRA MALAYSIA**

# *ALLELOPATHIC ACTIVITY OF Sida cordifolia L. METHANOL LEAF EXTRACT ON SELECTED NOXIOUS WEED SPECIES*

**HASSAN AHMED**

**FP 2018 28**

# ALLELOPATHIC ACTIVITY OF *Sida cordifolia* L. METHANOL LEAF EXTRACT ON SELECTED NOXIOUS WEED SPECIES

**By**

**HASSAN AHMED**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Doctor of Philosophy**

**January 2018**

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**DEDICATION**

**TO MY GERM,**

**HUSSAIN**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

**ALLELOPATHIC ACTIVITY OF *Sida cordifolia* L. METHANOL LEAF EXTRACT ON SELECTED NOXIOUS WEED SPECIES**

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**January 2018**

**Chairman : Professor Abdul Shukor Juraimi, PhD**
**Faculty : Agriculture**

Growing concern regarding increase in herbicide resistance weed species due to extensive use of synthetic herbicides which promote soil contamination and pose pollution to our environment, hence, an alternative weed control strategy using natural herbicide was adopted. *Sida cordifolia* is a herb belonging to the family *Malvaceae* and grows throughout the tropical and sub-tropical regions. It invades and colonize a mass of land. The allelopathic effect of *S. cordifolia* leaf methanol extract were evaluated based on experiments conducted in laboratory, glasshouse and field condition to determine germination, growth, physiological and biochemical activity on weed plants namely *O. sativa* (weedy rice), *A. conyzoides, A. gangetica, E. crus-galli, E. colona* and *P. hysterophorus*. Sample of *S. cordifolia* was collected from wild area in Nigeria and prepared to powder. Extraction was conducted using 100 g of *S. cordifolia* leaf powder into 2 litres 70% methanol. Concentration levels were prepared from stock to obtain 10, 20. 30, 40, 50, 75 and 100 % while distilled water was used as the control. Germination percentage, shoot and root lengths were reduced with increase in concentrations of the *S. cordifolia* extract. A declined in germination from control was 3.33, 60.0, 72.0, 79.12, 91.26 and 100 % occurred in *O. sativa, A. conyzoides, A. gangetica, E. crus-galli, E. colona* and *P. hysterophorus,* respectively. Greater phytotoxicity was observed in *P. hysterophorus* across the parameters while roots were severely inhibited. Concentrations of Sida crude extract 1.5, 2.25 and 3 g $L^{-1}$ were sprayed to *O. sativa, A. conyzoides, E. crus-galli* and *P. hysterophorus* seedlings at 4, 6 and 8 leaves growth stages at 12 L $ha^{-1}$ with a hand atomizer. Distilled water was sprayed on control treatments. Data recorded revealed that the leaf extract had effect on plant height, total dry matter and leaf area while slight changes in chlorophyll content and photosynthesis rate were recorded. Transpiration rate and stomatal conductance showed high significant ($P \leq 0.05$) response at different growth stages and it appears more pronounced at low growth stage across species. Phytotoxicity follow order *P.*

*hysterophorus* > *A. conyzoides* > *O. sativa* > *E. crus-galli*. In the field experiment organo-silicone surfactants (Silwit HS 614) was incorporated with the leaf methanol extract concentrations (3, 6 and 9 g $L^{-1}$) to improve penetration and enhance performance while distilled water was sprayed on control. We investigated morphological, physiological and biochemical response of *A. conyzoides* and *O. sativa* grown under field condition in order to evaluate the degree of phytotoxic effect of foliar spray of *S. cordifolia* leaf methanol extract. Plant height and shoot dry weight were slightly greater in *O. sativa* while leaf area decreased and consequently resulted in reduction of growth. *Ageratum conyzoides* exhibited a significant reduction in chlorophyll contents, carotenoids, chlorophyll fluorescence, peroxidase, catalase and proline. Seven days after the second foliar spray of the *S. cordifolia* extract leaves yellowing and scorching in *A. conyzoides* was observed which lasted for a period of 10 days but subsequently subsided, grow and recovered rapidly. The reduction in plant biomass was observed to be related todecreased in chlorophyll content. Activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were stimulated by foliar spray of *Sida cordifolia* methanol leaf extract (SLE) in a concentration dependent pattern. *Ageratum conyzoides* maintain comparatively low stress tolerance to SLE compared to *O. sativa*. Generally phytotoxicity effect of the Sida extract was pronounced in *A. conyzoides* and *P. hysterophorus* (Dicots). This could be due the inherent difference, assisted penetration by Surfactant (Silwit 614) and leaf surface area which enable better absorption and penetration of active allelopathins present in the *S. cordifolia* extract. Ultra Performance Liquid Chromatography revealed a high concentration of rutin while traces of other allelopathins (Quertecin, DL-phenylalanine, *P*- anisic acid, palmitic acid, stearic acid and syringic and acids) were detected. We suggest further investigations on bioassay in the field on other native weed species to address ecological relevance of our findings, extensive studies on phytochemical analysis of the plant extract including explorations in formulation techniques using surfactants and potency assessment in-situ for their effect on sustainable weed management in agriculture.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENILAIAN ALLELOPATI DAN AKTIVITI DARIPADA EKSTRAK METANOL DAUN *Sida cordifolia* KE ATAS SPESIS RUMPAI BERBAHAYA TERPILIH**

Oleh

**HASSAN AHMED**

**Januari 2018**

**Pengerusi : Profesor Abdul Shukor Juraimi, PhD**
**Fakulti : Pertanian**

Peningkatan kesedaran terhadap kewujudan racun rumpai rintang, penggunaan bahan kimia sintetik berlebihan dan pencemaran tanah terhadap persekitaran, telah mewujudkan kawalan alternatif bagi rumpai menggunakan racun rumpai semulajadi. *Sida cordifolia* adalah sejenis tumbuhan herba di bawah famili Malvaceae dan tumbuh di seluruh kawasan tropika dan sub tropika. Ia menyerang dan tersebar di kawasan yang luas. Kesan allelopati daripada ekstrak methanol daun *S. cordifolia* telah dikaji di dalam makmal, rumah kaca dan kajian lapangan untuk menentukan kadar percambahan, pertumbuhan, aktiviti fisiologi dan biokimia keatas rumpai *O. sativa* (padi angin), *A. conyzoides, A. gangetica, E. crus-galli, E. colona and P. hysterophorus.* Ektrak *S. cordifolia* telah diperolehi daripada kawasan liar di Nigeria dan telah di sediakan dalam bentuk serbuk. Pati kepekatan telah disediakan daripada stok ekstrak *S. cordifolia* (100 g $L^{-1}$) untuk menghasilkan siri kepekatan 10, 20, 30, 40, 50, 75 dan 100 %, sementara air suling adalah sebagai kawalan. Peratus percambahan, panjang pucuk dan akar telah berkurang secara berkala dengan peningkatan kadar kepekatan ekstrak daun *S.cordifolia.* Kadar percambahan telah berkurangan berbanding faktor kawalan (air suling) sebanyak 3.33, 60.0, 72.0, 79.12, 91.26 and 100 % *O. sativa, A. conyzoides, A. gangetica, E. crus-galli, E. colona and P. hysterophorus.* Kadar ketoksikan yang lebih tinggi telah direkod ke atas semua parameter tumbuhan *Parthanium* kecuali kadar pertumbuhan akar telah disekat dengan sepenuhnya. Rawatan disembur menggunakan penyembur tangan pada kadar 12 L $ha^{-1}$ ke atas biji benih diperingkat 4, 6 dan 8 daun (GS4, GS6 dan GS8). Air suling disembur pada rawatan kawalan. Data menunjukkan ekstrak daun tidak mempunyai kesan terhadap ketinggian pokok, jumlah berat kering dan keluasan daun, manakala terdapat sedikit kesan terhadap kandungan klorofil dan kadar fotosintesis. Proses transpirasi dan konduksi stomata menunjukkan kesan signifikasi yang tinggi ($P<0.05$) pada peringkat pertumbuhan yang berbeza dimana lebih ketara

pada peringkat pertumbuhan yang lebih rendah untuk setiap spesis. Kadar ketoksikan adalah mengikut urutan berikut, *P. hysterophorus* > *A. conyzoides* > *O. sativa* > *E. crus-galli*. Di dalam kajian lapangan, 'surfactants' silicon-organo (Silwit HS 614) telah digabungkan bersama ekstrak methanol daun untuk meningkatkan kadar penembusan dan prestasi. Kajian berkenaan kadar ketoskikan semburan ekstrak methanol daun *S.cordifolia* telah dijalankan di lapangan untuk melihat kesannya terhadap morfologi, fisiologi dan tindakan biokimia *A.conyzoides* dan *O.sativa*. Ukuran ketinggian pokok dan berat kering pucuk adalah lebih tinggi di dalam *O.sativa*, manakala keluasan daun berkurang dan menyebabkan pengurangan pertumbuhan pokok. *Ageratum conyzoides* menunjukkan kadar signifika penurunan kandungan klorofil, karatenoids, 'fluorescence' klorofil, peroksida, katalis dan prolin. Selepas 7 hari semburan kedua ekstrak daun *S.cordifolia* keatas *A.conyzoides*, kesan menunjukkan perubahan warna diikuti nekrosis dan kesan kebakaran daun yang berlaku selama 10 hari dan kemudiannya berhenti dan kembali tumbuh dengangn cepat dan baik. Kadar pengurangan di dalam biomas tumbuhan berkadaran lanasung dengan pengurangan kandungan klorofil. Kadar aktiviti enzim antioksida SOD, CAT dan POD dipengaruchi/didorong dan diransang oleh semburan daun SLE mengikut kadar semburan. *Ageratum conyzoides* masih mengekalkan kadar tekanan toleransi rendah berbanding (padi angin). Secara keseluruhannya, kesan ketoksikan ekstak daun *S.cordifolia* adalah lebih ketara keatas *A. conyzoides* dibandingkan dengan *O.sativa* (padi angin) disebabkan perbezaaan yang wujud, kadar penembusan oleh 'Surfactant' (Silwit 614) dan inas permukaan daun yang membolehkan penyerapan dan penembusan bahan aktif 'allelopati' yang terdapat di dalam ektsrak padi kadai *S. cordifolia* dengan lebih baik. Ujian 'Prestasi Ultra Kromatografi Cecair' merekodkan kadar konsentrasi rutin yang tinggi ditentukanmanakale allelopathic lani empat kompaun (catechol, vanillic, syringic dan chlorogenic acids). Di masa hadapan boleh dipertimbangkan kajian berkaitan ekstrak formulasi nano-emulsi dan alelokimia untuk meningkatkan prestasi.

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I certify that a Thesis Examination Committee has met on 17 January 2018 to conduct the final examination of Hassan Ahmed on his thesis entitled "Allelopathic Activity of *Sida cordifolia* L. Methanol Leaf Extract on Selected Noxious Weed Species" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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| | |
|---|---|
| DNA | Deoxyribonucleic acid |
| MEP | Methylerythritol phosphate |
| LAI L | Leaf area index |
| PSII | Photosystem II |
| HPPD | Hydroxylphenylpyruvate dioxygenase |
| ROS | Reactive oxygen species |
| CAT | Catalase |
| MDA | Monodehydroascorbate reductase |
| POD | Peroxidase |
| SOD | Superoxide dismutase |
| GPX | Guaiacol peroxidase |
| GSH | Glutathione reductase |
| SSR | Simple sequence repeat |
| HPLC | High performance liquid chromatography |
| LC-MS | Liquid chromatography mass spectrum |
| UPLC | Ultra performance liquid chromatography |
| SQD | Single quadrupole detector |
| ANOVA | Analysis of variance |
| RT | Retention time |
| Ppm | Part per million |
| LC-MS-QTOF | Liquid chromatography mass spectrum quadrupole time-of-flight |
| SAS | Statistical analysis software |
| LSD | Least significant difference |
| SPAD | Soil plant analyses development chlorophyll meter |
| *A* | Net photosynthetic rate |
| *g* | Stomata conductance |
| *E* | Transpiration |
| GS | Growth stage |
| SPP | Species |
| RDW | Root dry weight |
| SDW | Shoot dry weight |
| LA | Leaf area |
| Fm | Maximum fluorescence |
| Fv | Variable fluorescence |
| Fo | Minimum fluorescence |
| g | Gram |
| Kg | Kilogram |
| SLE | *Sida cordifolia* methanol leaf extract |
| µmole | Micromole |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

Allelopathy is derived from the Greek words *allelon* "mutual" and pathos "to suffer". It is therefore referred to deleterious and beneficial biochemical interactions between plants. Plant existence in the natural environment is subject to the effect of numerous beneficial and detrimental relationship and interactions with the neighboring organisms within its ecosystem. May and Ash (1990) define allelopathy as an interference system in which chemical substances which stimulate or inhibit plant growth are released from live or dead plant material. Chemical interactions between plants of different species involve the release of phytochemicals called allelochemicals/allelopathins. In allelopathic interaction, dominant species liberates toxins which act on vulnerable or susceptible plant resulting in a beneficial advantage to the toxin donor plant. Plant species inhabiting the same niche tend to compete with each other for important natural resources such as water, sunlight, and nutrients within the vicinity.

Allelochemicals are particularly meaningful as natural agrochemicals with the potential to protect our environment from pollution and maintain better ecosystem balance. These chemicals are mainly classed as secondary metabolites present in almost all plant tissues which may be released to the environment via leaching, decomposition of plant residue and volatilization. However release of such chemical compounds adversely affects germination and growth of plant through their interference in cell division, energy metabolism, mineral uptake and biosynthetic processes (Rice, 1984). The readily visible effect of allelopathy includes inhibited germination, seed swollen and darkened, necrosis of root, discoloration, shoot length, radicle/root length, reduced dry weight and reproductive ability (Niakan and Saberi 2009), leading to significant reduction in photosynthesis and other plant functions (Hussain and Reigosa, 2011). These morphological effects are secondary manifestation of primary activity caused by wide range of allelochemical compounds acting at a molecular or cellular level to the recipient plants (Peng *et al*., 2004; Singh *et al*., 2006).

Despite numerous studies on allelopathy interactions among different plant species yet explicit mechanism of activity of allelopathin remains a challenge, many findings on the allelopathic effect of plants were mostly conducted with crude extract of aerial or root organ comprising of many phytochemicals of unknown species. These phytochemicals were known to exhibit a broad and diverse spectrum effect on germination, membrane functions, cell elongation, photosynthesis, respiration, synthesis of certain compounds, mineral uptake, carbon flow, water relation, stomatal function, plant metabolism and cell death. Knowing the mechanism of

action of allelopathin as well as identification of the relative active compounds present in *Sida cordifolia* will enhance our ability to explore and ascertain its potential use on weed management and a template for natural herbicide. This study in intended to unveil the important mechanisms of growth inhibition related to germination and growth, physiological and biochemical activity of noxious weed species at different growth stages.

*Sida cordifolia* is a herb belonging to the family *Malvaceae*. It is found throughout the tropical and sub-tropical plains of India, Sri Lanka, Malaysia, North America and to the West and East Africa including Nigeria, Niger, Cameroun, Kenya and Sudan. Common names include flannel weed, heart-leaf sida and country mallow. It has naturalized throughout the world and have been declared a class B and C weed by Weeds branch, Darwin (Pitt, 2002). Interactions between plants may present a unique competition and invasive succession in an environment. The interaction between plants (allelopathy) brings about suppression mechanism among species and hence associated with the species invasiveness and succession (Callaway and Ridenour, 2004). Although simple laboratory bioassay or glasshouse experiment can be used to examine the potential allelopathic effect of a plant species, however, field studies are necessary to determine the allelopathic efficacy of a donor plant on the receiver specie in a natural environment.

## 1.2 Justification

Due to the incessant application of synthetic herbicides in the agricultural system coupled with a rapid increase in herbicide-resistant weed species across the globe, much attention has been focused on an alternative weed management method/techniques including allelopathy which is eco-friendly and will reduce reliance on synthetic herbicide. *Sida cordifolia* is used extensively in traditional medicine and commonly studied on its medicinal value but there is lack of information on its herbicidal potential. Plant invasion of other neighboring plants in a natural habitat is an indication of a potential allelopathic activity. Visual observation of site invaded by *S. cordifolia* displays a drastic decrease in the preponderance of indigenous plants species. This plant is observed to colonize and invade a mass of land and dominate other species perhaps via seed proliferation, leaves or root exudation of phytotoxins as one of the proposed mechanisms to gain advantage over indigenous species. Hence this study was an attempted to examine the allelopathic potential of *S. cordifolia*.

## 1.3 Objectives

The study aimed to investigate the inhibitory/stimulatory effect of methanol leaf extract of *S. cordifolia* on germination, growth and biochemical activity of some selected invasive weed species in Malaysia.

The specific objectives of the study were:

1. To identify the phytochemical constituents of methanol leaves and root extracts of *S. cordifolia*.

2. To determine the effect of methanol leaf extract of *S. cordifolia* on germination and seedling growth of *Echinochloa crus-galli, E. colona, Oryza sativa, Asystasia gangetica, Ageratum conyzoides* and *Parthenium hysterophorus*.

3. To evaluate growth and physiological effect of *S. cordifolia* leaf extract on the noxious weed species under glasshouse condition.

4. To determine growth, physiological and biochemical effect of *S. cordifolia* leaf extract on the experimental weed species under field conditions.

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## LIST OF PUBLICATIONS

Ahmed, H., Juraimi, A.S., Hamdani, M.S.A., Omar, D. and Rafii, M. Y (2016) Herbicidal efficacy of leaf methanol extract of *Sida cordifolia* on germination of *Ageratum conyzoides* and *Parthenium hysterophorus*. Proceedings 7th International Agriculture Congress, Pp273- 278

Ahmed. H., A. S. Juraimi and M. S. A. Hamdani (2016). Introduction to Robotics Agriculture in Pest Control: A review, *Pertanika Journal of Scholarly Research Review* 2(2): 80- 93.

Ahmed. H., Juraimi, A.S., Hamdani, M.S.A., Omar, D., Rafii, M. Y and Aslani, F (2017) Comparative phytotoxic effects of aerial and root aqueous extracts of *Sida cordifolia* L. on germination and seedling vigour performance of lettuce, tomato and carrot. *Bangladesh Journal of Botany*, Supplementary, 46(1): 323-328.

Ahmed. H, Abdul Shukor Jurimi, Muhammad Saiful Ahmad Hamdani, Dzolkifli Omar, Muhammad Yusof Rafii, Mallappa Kumara Swamy, Uma Rani Sinniah, Mohd. Sayeed Akhtar (2017) Botany, Chemistry and Pharmaceutical Significance of *Sida Cordifolia*- A Traditional Medicinal Plant, Chapter. (Accepted)

Ahmed. H., Juraimi, A.S., Hamdani, M.S.A., Omar, D. and Rafii, M. Y (2017) Physiological response of weeds induced by leaf methanol extract of *Sida cordifolia* (Linn). (Manuscript submitted)

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