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Preliminary Study on Allelopathic Effect from Chromolaena Odorata (Siam Weed) Leaves Extract Towards Vigna Radiata

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***Abstract*— *Chromolaena odorata* is a fast-growing perennial shrub that grows wild in Asia, Africa and South America. It forms dense stands that prevent the establishment of other plant species and suppress the plants by competing for nutrients and water, over-shading and allelopathy. The study conducted investigates the allelopathic effects of *C. odorata* leaf extract on the germination and growth rate of *Vigna radiata* (mung bean). Methanolic extract at concentrations of 20, 40, 60 and 80% were used in the study in order to compare the effectiveness of allelochemicals level in inhibiting the growth of germinated mung bean. The methanolic extract from the leaves exhibited a potent growth inhibitory effect. Inhibition of roots and shoots elongation of mung bean showed a concentration and percentage methanolic extract dependent. Roots and shoots elongations were inhibited at all concentration of methanolic extract. Treatment with 80% methanolic extract showed a greater effect on germination rate mung bean compared to other concentrations. Phytochemical analysis was conducted on the 80% of methanolic extract to confirm the presence of alkaloids, tannins, flavonoids, steroids, terpenoids and carbohydrates compounds. The presence of tannins, flavonoids, alkaloids and terpenoids speed up the inhibition on mung bean growth as well as its germination.**

Keywords: *Chromolaena odorata, allelochemicals, Vigna radiata*

I. INTRODUCTION

C. odorata or also known as Siam weed is a fast-growing perennial shrub, native to South America and Central America. It has been introduced into the tropical regions of Asia, Africa and the Pacific where it is an invasive weed. It forms dense stands that prevent the establishment of other plant species [1]. It can suppress crops and other plants by competing for nutrients and water, over-shading and allelopathy [2]. *C. odorata* leaves especially the young ones are toxic due to high levels of nitrate [3]. *C. odorata* is heliophile. It needs light to germinate and is suppressed when shaded by other plants. The plant grows on a wide range of solids, but not on flooded sites. Through symbiosis with vesicular-arbuscular mycorrhizae, it also can grow well on poor soils condition [4]. Under a *C. odorata* uncultivated, the soil structure improves and the pH and biological activity of poor soil increase. Reference [2] stated that *C. odorata can* tolerates mechanical injuries caused by slashing and burning, as it is able to form new shoots on the swollen part of the root. However, frequent injuries will reduce the plant's regenerative capacity [5].

C. odorata plant contains the allelochemicals which allow the plant to affect germination, growth, development, distribution and behaviour of neighbouring plants including weeds [6], [7], [8]. Allelochemicals are defined as any direct or indirect harmful or beneficial effect by one plant including microorganisms on another through production of chemical compounds that escape into environment [9]. The chemical can be found in root, stem, leaves, flowers and fruits, and inhibit root growth, shoot growth, germination percentage as well as nutrients uptake [10], [11]. The allelopathic effects are selective and vary with different trees since these plants will vary in the amount of original secondary metabolites and would release different amount of the phytotoxins.

Generally, leaves are the most powerful source of allelochemicals [12], [13], [14]. As mentioned by reference [15], the mode of action of allelochemicals can broadly be divided into indirect and direct action. Indirect action may include effects through alteration of soil property, its nutritional status and an altered population and activity of harmful or beneficial organisms like microorganism, insects and nematodes [16]. On the other hand, the direct mode of action which includes effects of allelochemicals on various aspects of plant growth and metabolism has received fairly wide attention [9], [16] if compared to the indirect action.

Vigna radiata (L.) Wilczek or mung beans have been grown in India since the ancient times. The plant is still grown in countries of Southeast Asia, Africa, South America and Australia. It was apparently grown in the United States since 1835 as the Chickasaw pea. The bean is also referred to as green gram, golden gram and *chop suey* bean. The beans are grown widely due its usage as a human food, green manure crop and forage for livestock [17].

II. MATERIALS AND METHODOLOGY

A. *Sample Harvesting, Handling and Storage*

Sample of *C. odorata* leaves was collected from the rural area of Alor Gajah, Melaka, Malaysia. Sample preparation was done base on method adapted from reference [18]. The leaves were cleaned and washed by using tap water before cut into small pieces of 1 cm x 1 cm and dried in drying oven (MEMMERT, Germany) for 2 days at 40°C. The dried leaves were further ground to a fine powder by using a laboratory blender (WARING, USA) and kept in a bottle wrapped with aluminium foil. Grounded sample was stored at 2°C until further usage.

B. *Extraction of C. odorata Leaves*

Extraction was conducted by using soxhlet extraction method (Gerhardt, UK). The extraction method used was as mentioned by reference [19] and [20]. Methanol (MERCK, Germany) at 99.8% purity was used as a solvent and further diluted into five different methanol concentrations (20%, 40%, 60% and 80%) in order to observe the effect of different solvent concentration on the growth of *V. radiata*. 15.5 g of *C. odorata* powder was extracted exhaustively by using 200 ml of solvent for 12 hours.

C. *Treatment on Vigna radiata*

Seeds of *V. radiata* were purchased from local store of Alor Gajah, Melaka, Malaysia. Only dense seeds were used in this study. Seeds were soaked with water for 4 hours obtain the maximum seed yield [21]. The medium for germination and growth of mung bean was based on study done by [18] and [20]. Whatman #1 filter paper was used as a base for the seeds germination in the Petri dish with each dish contained 3 seeds.

Seeds were incubated in the laboratory at 26 – 27°C. 10 ml of distilled water was added to retain the humidity of the filter paper while 2 ml of *C. odorata* extract (20%, 40%, 60% and 80%) was added to the seeds on day 4 which is after the root and shoots were formed. A control dish was prepared where the seeds were treated with methanol and water. The extract and methanol solution was added to the seeds at every 2 days. Germination rate was observed and measurement of shoots and roots length was taken by using yarn started from day 2. The measurement was conducted for 9 days. Study was done in triplicate.

D. *Phytochemical Screening*

Phytochemical tests was conducted on the 80% methanolic extract of *C. odorata* leaves by using standard qualitative methods as describe by [22]. The extract was tested on the presence of flavonoids, tannins, alkaloids, steroids, terpenoids and carbohydrates.

III. RESULTS AND DISCUSSIONS

A. *Allelopathic Treatment of V. radiata*

Allelopathic treatment is an environmental-friendly technique to identify the effects of allelochemicals on the growth and development of plants. In this study, the allelochemicals released from *C. odorata* were used as the test solution on germination rate study conducted on mung bean seeds. By exposing the seeds to allelochemicals, the inhibition effects on the roots and shoots elongation can be observed. Allelopathic treatment started on day 4 after mung bean being germinated and growth in order to allow the formation of roots and shoots of the seeds and later to observe the effect of each concentration of extract added as suggested by [23].

C. odorata leaves were chosen in this study because it is the most consistent producers of allelochemicals as mentioned by [24]. Reference [5] mentioned that phenols and alkaloids in the plant, particularly in the leaves, have an allelopathic effect which inhibits the germination of its own seeds and also the seedling development of other plants. Similar types of result are noted in this study that shows the leaf extract reduced germinability and caused slower rate of mung bean seed growth. Reference [25] stated that the slower rates of germination are considered to be the most important visible and reliable keys for the evaluation of allelopathic effect. In addition, reference [26] reported that the aqueous extract of *C. odorata* leaves had the highest inhibitory effect on germination of paddy and barnyard grass in comparison to the aqueous extract obtained from stem and root. They found out that the inhibitory effect on germination of barnyard grass was at the maximum level in the leaf extract at a higher concentration.

B. *Analysis of Roots and Shoots Elongation*

It is shown in the present study that the speed of germination was adversely affected in the mung beans seed treated with the leaf extracts of *C. odorata* in comparison to control. However, the decreasing point of the rate of germination was found to be much more prominent in 80% methanolic extracts treated sets and the effect was fully concentration dependent which agreed with the study conducted by reference [25].

The inhibition of the methanol extract from *C. odorata* leaves on the root growth was greater if compared to shoot growth. The results were shown in Figure 1, 2, .3 and 4. These results are in agreement with the results obtained by reference [20], which reported that the extracts of allelopathic plants had more inhibitory effect on root growth than on hypocotyls growth because root is the first organ to absorb the allelochemicals from the environment. Reference [27] studied that the capability of the *C. odorata* leaves extract to inhibit is higher than effect caused by leachate on the seed development.

The length and characteristic of roots and shoots were changed after the addition of the test solution on day 4 which showed the effects of allelochemicals on their growth and development. Their physiological activities were also altered drastically after the addition of the test solution and it is relative to the concentration of methanolic extract. 80% of methanolic extract showed the greatest physical changes on root and shoot if compared to the others. Allelochemicals affect the colour and structure of root and shoot by changing from green to black shoot while the root's colour became brown as agreed by reference [28]. The root systems were swollen and easier to split after affected by allelochemicals which is also comparable to the finding made by reference [28] and [18].

The effects of 20% and 40% methanolic extract of *C. odorata* on root and shoot elongation of mung bean are shown in Figures 1 and 2 below. Roots and shoots elongation of mung bean increased rapidly on day 3 of the treatment and decreased after the addition of test solution due to the inhibitory effect of the extract. Similar findings have been reported by reference [29] which discovered that even at the lowest concentration of extract still inhibit the growth of the wheat rootlet and shoot when compared to the control.

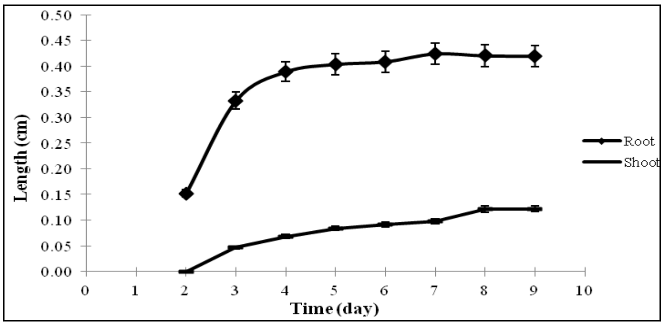

Figure 1. Effect of 20% methanolic extracts on root and shoots elongation.

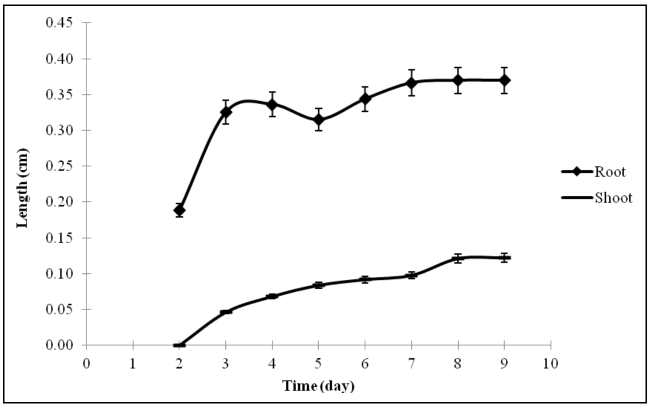

Figure 2. Effect of 40% methanolic extracts on root and shoots elongation.

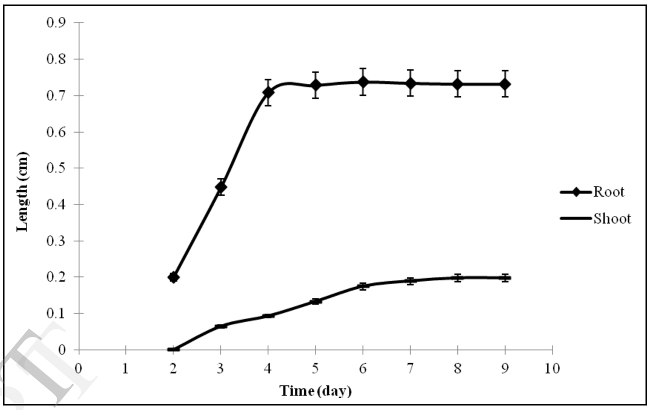

Figure 3. Effect of 60% methanolic extracts on root and shoots elongation.

Graph in Figure 4 below shows the effect of 80% methanolic extract of *C. odorata* on the germination rate of mung bean. It shows that 80% methanolic extract reduced the germination rate starting on day 5. Small increment of roots and shoots elongation clearly seen on day 5 to day 6 which point out that the higher methanolic extract will cause faster effect on the roots and shoots elongation and greater allelopathic effect on the root and shoots elongation of germinated mung bean seeds which is equivalent with the result obtained from reference [30]. Their results showed that the increase of concentrations of leaf extract exhibit the lowest root length. Allelopathic effect increased with the increasing concentration of *C. odorata* extracts [26].

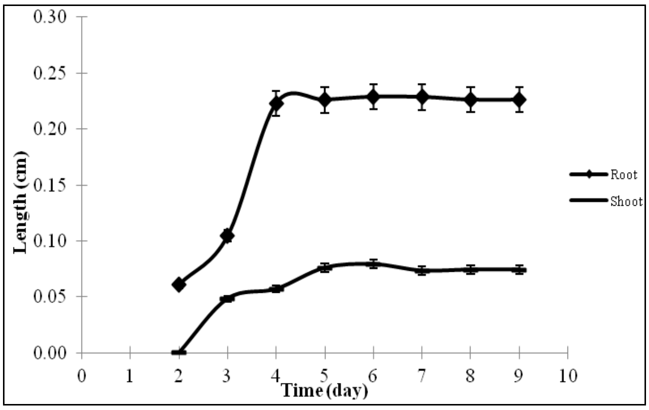

Figure 4. Effect of 80% methanolic extracts on root and shoots elongation.

Root elongation of mung beans treated with 20%, 40%, 60% and 80% methanol concentration, water and methanol was shown in Figure 5. It shows that 80% methanolic extract caused the slowest rate in mung bean germination due to the highest concentration of extract. Similar effect was observed on mung bean treated with 60% of methanolic extract. Reference [23] found that root growth may have been affected more than stem and leaves because roots were in continuous contact with the extracts which is similar to the results in this study.

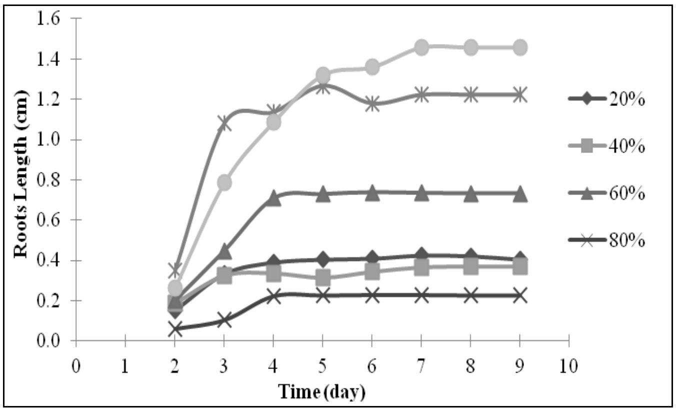

Figure 5. Effect of different methanolic extract and control solutions on root elongation of mung bean.

Shoots elongation of treated mung bean seeds are shown in Figure 6 below. Water control showed that the shoots elongation of mung bean grow healthier if compared to the condition when it was treated with 99.8% methanol. The treated mung bean with 20% methanolic extract showed the elongation of shoots was inhibited on day 8. Meanwhile, the elongation of shoots were inhibited completely on day 6 when it was treated with 80% methanolic extract due to higher amount of terpenoids released as studied by reference [29].

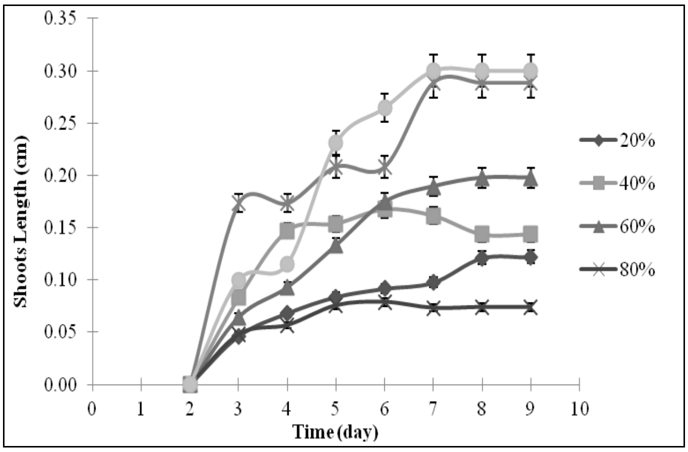

Figure 6. Effect of different methanolic extract and control solutions on shoot elongation of mung bean.

C. *Analysis of Phytochemical Constituent*

Phytochemical screening was conducted only on 80% methanolic extract due to its greater allelochemicals effects in inhibit the roots and shoots elongation of germinated mung beans. Qualitative analysis was carried out for methanolic extract of the leaves of *C. odorata* showed the presence of five major groups of phytochemical constituents. The result obtained is summarized in Table 4.1. Phytochemical screening of the plant leaves exposed the presence of flavonoids, tannins, alkaloids, steroids, terpenoids and carbohydrates were present in *C. odorata* extract. Flavonoids are mainly found in the leaves of plants [31].

TABLE I. PHYTOCHEMICALS ANALYSIS IN 80% OF METHANOLIC EXTRACT OF *C. ODORATA*

Phytochemical	**Methanolic Extract**	
	A	**B***
Flavonoids	Present	Present
Alkaloids	Present	Present
Tannins	Present	Present
Steroids	Present	Present
Terpenoids	Present	Present
Carbohydrates	Present	Not tested

A – Present study **B** – Studied by reference [32]

The findings revealed that the crude extracts contain flavonoids, tannins, alkaloids, steroids, terpenoids and carbohydrates. The results obtained were supported with the phytochemical similarity of compounds reported by reference [32] which also revealed the presence of alkaloids, flavonoids, tannins, steroids and terpenoids in methanolic extract of *C. odorata*. They found that alkaloids compounds were detected only in methanolic extract but not in the aqueous extract. The presence of phytochemical constituents in 80% methanolic extract is greater compared to 20%, 40% and 60% methanolic extract. The higher of methanol concentration being used, the greater phytochemical constituents released from *C. odorata* leaves and thus, the greater inhibitory effect on germination and growth of mung beans.

D. *Effect of Phytochemical Constituents*

Phytochemical constituents released in 80% methanolic extract of *C. odorata* leaves interact each other to accelerate the effect of allelopathic on the germination of mung bean seeds. Alkaloids, terpenoids, tannins, flavonoids and phenolic compounds presence in the extract provide allelochemicals mechanism that inhibits the growth of mung bean germination. Results obtained by reference [6] found that in the vitro experiments with more than 70 alkaloids indicate that most alkaloids are toxic or inhibitory to more than one group of organisms including plant seedlings, bacteria, insects and mammals. While reference [33] found that the higher amount of alkaloids can inhibit the cell division and cell wall formation.

The presence of terpenoids in the extract will cause an interfering the mitotic cell of the plant and contribute to seed germination inhibition [29]. Tannins and flavonoids are the group of phenols that is also known as polyphenols which have the ability to inhibit seed germination because they are likely to have biological toxicity [34].

Result in this study showed that lateral root development was greatly reduced by *C. odorata* terpenoids. The number of lateral root initials is less than in control roots and most of these laterals never expanded very far into the cortical region as exposed by reference [35]. Only with rather low concentrations of toxins will cause the lateral roots ever rupture through the epidermis. In control roots, after a similar times interval, many laterals will extend through the epidermis.

IV. CONCLUSIONS

This study shows the effect of allelopathy mechanism from *C. odorata* leaves extract on the germination of mung beans seeds. The inhibition was clearly seen on the roots and shoots elongation of germinated mung bean seeds. The growth inhibition activity of methanolic extract from *C. odorata* provides the opportunity to explore on the new compounds from the plant itself. The phytochemical screening on the extract proved that there were presence of tannins, flavonoids, alkaloids, terpenoids, steroids and carbohydrates which aid in the inhibition on the roots and shoots elongation of germinated mung beans.

This investigation thus concludes that by virtue of its strong inhibitory effect, the invasive weed *C. odorata* has the potential to interrupt regeneration processes of mung bean and other species of plant by decreasing the germination and reducing early growth rates as well as the potential of using allelopathic species to suppress the growth of weeds. It can further be used as a tool to formulate new eco-friendly bio-herbicides for weeds control in agro-ecosystems and natural ecosystems.

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