

Allelopathic Effects of *Imperata Cylindrica* Aqueous Extract on the Germination of *Cucumis Sativus* and *Lolium Perenne*



Mohamed Ali A. Salim, Alona Cuevas Linatoc, Suzi Salwah Jikan, Shajarahtunnur Jamil, Muhammad Sohail Latif

Abstract: In this study, the allelopathic effect of *Imperata cylindrica* has been investigated. Aqueous extracts of *Imperata cylindrica* were tested at concentrations of 31.25, 62.5, and 125 mg/ml to study different parameters on *Cucumis sativus* and *Lolium perenne*. Various factors were studied to determine the inhibition and growth of root and shoot length, percentage germination, and fresh and dry weight. Results indicated that the aqueous extract of *Imperata cylindrica* contain allelochemicals which may contribute to its invasiveness and extreme competitiveness. The results of this study show that *Imperata cylindrica* contains allelochemical compounds particularly in root and shoot extracts that might be the potential candidates for future investigations for the development of herbicides based on its secondary metabolites and their allelopathic inhibitory effects. Further research is needed to investigate the allelopathic effect of *Imperata cylindrical* aqueous extracts under field conditions against the associated weed species and elucidation of the mechanism of inhibition involved

Keywords : Allelochemicals, Allelopathy, *Imperata cylindrica*, weed control.

I. INTRODUCTION

Allelopathy can be defined as the certain secondary

metabolites synthesized by plants and microorganisms, released to the environment causing inhibition of the growth of neighbor plants and microorganisms [1, 2]. The direct allelopathic interactions can influence plant success within an ecosystem through a variety of mechanisms including inhibition of germination, limit growth, or hinder nutrient uptake of target plants [3, 4]. Indirectly, allelopathic chemicals may inhibit the activity of mutualistic bacteria and fungi in the soil, also limiting the success of the target plant [5, 6]. Since their field application in 1940, there was a gradual increase in the dependency of herbicides that replaced all other approaches of weed control such as mechanical, cultural and manual due to cost-effective and adequate weed management by using herbicides [7]. Despite the large-scale adaptation by the farmers and tremendous success, the continuous and heavy applications of herbicide led not only to deteriorate the ecosystem but also posed severe health hazards and evolution of weed populations resistant to the herbicides [8]. It was reported that 255 weeds from 92 crops have become resistant to 163 herbicides around the world [9]. Moreover, serious threats are increasing around the world on the potential carcinogenic and toxicological consequences of herbicides. Due to very large scale and frequent applications, the herbicide residues including their primary metabolites are disturbing the ecosystem after accumulation in the ecosystem and disturbing the food chain, leading to contamination of soil and water bodies and, hence, posing a serious threat to animal and human health [10]. The present situation demands new strategies for solutions to these chronic issues. In this context, allelopathy is considered as a multi-faceted science whose one aspect is to utilize the naturally occurring allelochemicals for effective weed management. The great benefit of such compounds is that the plants that can be directly applied as herbicides naturally produce them. These allelochemicals are efficient, cost-effective and eco-friendly [11]. There are many ways for effective weed control by using such naturally occurring phytotoxic products like mulches, cover crops or direct spray as organic herbicides [12]. In literature, there are several research reports regarding the allelopathic activity of various plants. These naturally occurring allelochemicals have been reported for their easy extraction and use in spray form just like herbicides [13]. The problem of weed control in crops is increasing in modern "organic farming" as the application of pesticides and herbicides is reducing in response to decreasing environmental degradation and negative impacts on human health [14].

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* Correspondence Author

Mohamed Ali A. Salim, Department of Technology & Natural Resources, Faculty of Applied Sciences & Technology, Universiti Tun Hussein Onn Malaysia (UTHM), KM1 Jalan Panchor, Pagoh Educational Hub, Pagoh, Muar, Johor, Malaysia. Email: ben_ganem@yahoo.com

Alona Cuevas Linatoc*, Department of Technology & Natural Resources, Faculty of Applied Sciences & Technology, Universiti Tun Hussein Onn Malaysia (UTHM), KM1 Jalan Panchor, Pagoh Educational Hub, Pagoh, Muar, Johor, Malaysia. Email: alona@uthm.edu.my

Suzi Salwah Jikan, Department of Technology & Natural Resources, Faculty of Applied Sciences & Technology, Universiti Tun Hussein Onn Malaysia (UTHM), KM1 Jalan Panchor, Pagoh Educational Hub, Pagoh, Muar, Johor, Malaysia. Email: suzi@uthm.edu.my

Shajarahtunnur Jamil, Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia. Johor Bahru, Johor, Malaysia. Email: shaja@kimia.fs.utm.my

Muhammad Sohail Latif, Department of Technology & Natural Resources, Faculty of Applied Sciences & Technology, Universiti Tun Hussein Onn Malaysia (UTHM), KM1 Jalan Panchor, Pagoh Educational Hub, Pagoh, Muar, Johor, Malaysia. Email: slatif37@gmail.com

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The natural allelochemicals have low environmental risk as compared to synthetic chemicals and lower risk of resistance in the environment [15].

Allelopathic effects on native plants have been found in invasive species such as *Centaurea diffusa* which releases 8-hydroxyquinoline and *Solidago canadensis* which releases flavons, phenolics, and saponins [16, 17]. Allelopathy may function in the weed control as an element of an integrated strategy due to its selective nature. So, this study aimed at the investigation on the effect of *Imperata cylindrica*, containing the active allelopathic compounds, on the processes of seed germination, growth, and biomass of certain widely spread crop weeds. The *Imperata cylindrica* extracts were applied for bio-control of 4 different species of weeds [18].

Imperata cylindrica (L.) is a noxious weed that poses a serious threat to sustainable agriculture and global biodiversity [19]. Although it is reported to possess a variety of bioactive and medicinal principles, it has been utilized rarely for therapeutic and commercial purposes [20].

II. MATERIALS AND METHODS

A. Collection of Plant Material

Imperata cylindrica whole plant was collected from Batu Pahat, Johor, Malaysia. The plant material was identified in the herbarium of Biology Department, Faculty of Science, Universiti Kebangsaan Malaysia (UKM), Bangi, Selangor. The plant material was kept at the room temperature under shade for drying up to 30 days until further experiments.

B. Aqueous Extraction

The aqueous extraction method was used to obtain the crude extract of *Imperata cylindrica*. Different weights (3.125, 6.25 and 12.5 g) of above-ground parts (shoot consisting of stem/leaves/inflorescence) and below-ground parts (root consisting of root and rhizomes) of *Imperata cylindrica* were mixed with 100 ml distilled water in a 250 ml flask. The flask was covered with aluminum foil and kept at room temperature (28 ± 2 °C) for 24 h. on a shaker at 100 rpm. The mixture was filtered by using Whatman filter paper No. 1. The extract thus obtained was applied to *Lolium perenne* and *Cucumis sativus* (L.) to determine the allelopathic potential of *Imperata cylindrica* [21]

C. Phytogrowth Inhibitory Activity

Phytogrowth inhibitory activity of *Imperata cylindrica* extracts was determined by seed germination, radical and shoot growth, dry and fresh weight of treated seeds. Firstly, the seed surfaces were sterilized by soaking in 0.3 % sodium hypochlorite for 5 min followed by placing them in sterile distilled water. This step was repeated three times. Then, 10 seeds of selected (treated) weeds were placed on 9.0 cm diameter sterile Petri-dishes with Whatman filter paper No.1. in three replicates (Figure 1). Each plate was treated with 5 mL of the test extracts at different concentrations. Glyphosate (1ml/75 ml. distilled water) was used as a positive control and distilled water as a negative control [22]. Petri dishes were placed in a dark room at 28 ± 2 °C for 7 days.



Figure 1. Seed germination of selected weeds after treatment with test extracts of *Imperata cylindrica*

III. RESULTS AND DISCUSSION

Direct or indirect stimulation or inhibition of one plant on another through the release of chemical compounds into the environment is referred to as allelopathy. Allelopathic interactions involve the inhibition of plant growth and cell division by allelochemicals. Information about the allelopathic effects of water extracts of different parts of these weeds on the germination and seedling of *Imperata cylindrica* is scarcely available. To examine the effect of different concentration of water on the germination of weeds, an analysis was performed on percentage germination, wet and dry weight, root and shoot lengths.

A. The comparison of the effect of root and shoot water extracts of *Imperata cylindrica* on percentage germination of *Cucumis sativus* and *Lolium perenne*

Aqueous extract of *Imperata cylindrica* exhibits an allelopathic effect on % germination of *Cucumis sativus*. Root and shoot extracts have 0 percentage germination in a positive control environment of glyphosate. Around 70 % of normal germination was shown by root and shoot extracts in the negative control of distilled water. Root extract shows an increase of approximately 15 % germination as compared to shoot extract at the concentration level of 125 mg/ml. 20 % germination is increased by root extract in comparison to the shoot extract at 62.5 mg/ml concentration. The highest germination is shown at the concentration of 31.25 mg/ml root extract. This shows that approximately a 20 % rise in germination level is depicted by root extract at 3 different concentration levels in contrast to shoot extract. The % germination of root and shoot extract is more likely towards the negative control of distilled water. While an exception has been shown by root extract at the concentration of 31.25 mg/ml (Figure 2 (a)).

A comparison was performed to study the level of increase or decrease in % germination, root and shoot length, wet and dry weight by examining the effect of root and shoot water extracts of *Imperata cylindrica* on the germination of *Lolium perenne*. The range of positive and negative controls of % germination lies between 0 - 90 % approximately. The concentration of 125 mg/ml shows that % germination in root extract is around 50 % while shoot water extract has germination around 30 %. So, a significant increase of 20 % germination is shown by root extract as compared to shoot extract.

Similarly, 31.25 mg/ml concentration shows that there is a 10% increase in the germination of root extract as compared to the shoot extract. While contradictory behavior has been shown by shoot water extract at the concentration of 62.5 mg/ml. The % germination is more in the sample treated by shoot extract, that is, ~60 %, while it has decreased from ~50 % to 10 % in the sample treated by the root extract (Figure 2 (b)).

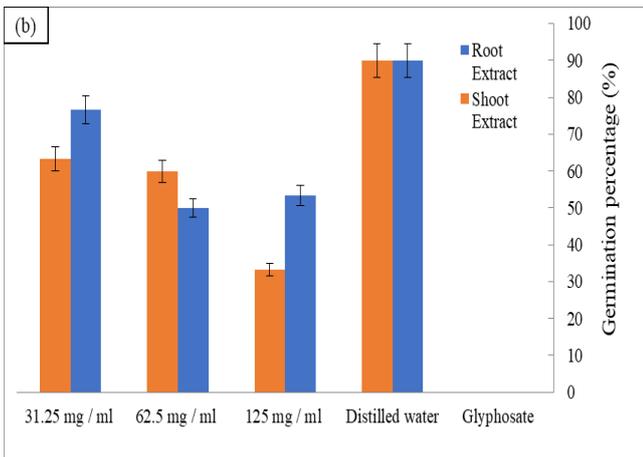
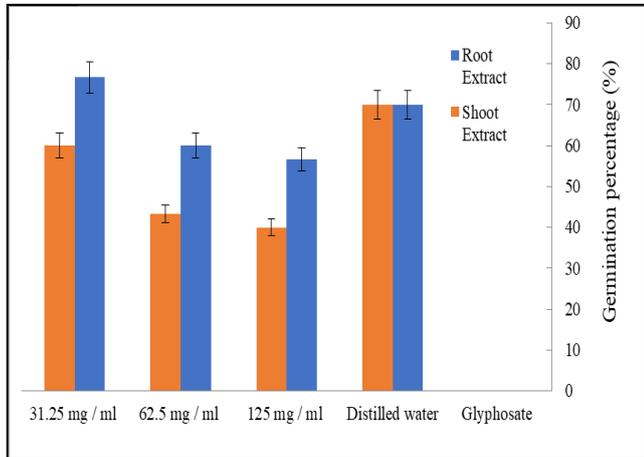


Figure 2. The comparison of the effect of root and shoot water extracts of Imperata cylindrica on percentage germination of (a) Cucumis sativus and (b) Lolium perenne

B. The comparison of the effect of root and shoot water extracts of Imperata cylindrica on root length of Cucumis sativus and Lolium perenne

The comparison between the root and shoot water extracts in terms of root length (cm) of Cucumis sativus is analyzed in Figure 3 (a). Glyphosate and distilled water are used as the positive and negative controls that produced the root lengths between 0 and 6 cm, respectively. At the concentration of 125 mg/ml, there is an increase of 0.5 cm root length in root extract as compared to shoot extract. While no significant increase in root length was observed in root and shoot extracts at 62.5 mg/ml, which shows an equal root length of approximately 2.2 cm. A slight difference of 0.5 cm root length increase was measured in root extract (~5cm) as compared to shoot extract (~4.5cm). Overall, a significant difference in root length was measured at the concentration of 31.25 mg/ml and 62.5 mg/ml. Also, the highest concentration

level of root length is relatively closer to the negative control of distilled water.

To examine the effect on the seed germination of Lolium perenne by Imperata cylindrica root and shoot water extracts, a comparison was performed on root length. The range of positive and negative controls of root length lies between 0-4 cm approximately. The concentration of 125 mg/ml shows that root water extract has ~1cm length while shoot water extract has a root length of 0.75 cm. So, there is a slight difference between these two root lengths. While at 62.5 mg/ml concentration, shoot water extract has a root length of 1.4 cm while root water extract has 0.75 cm, root length. 0.65 cm difference has been observed by shoot water extract and root water extract. The concentration of 31.25 mg/ml shows that root length in root water extract and shoot water extract are in close approximation to each other (Figure 3 (b)).

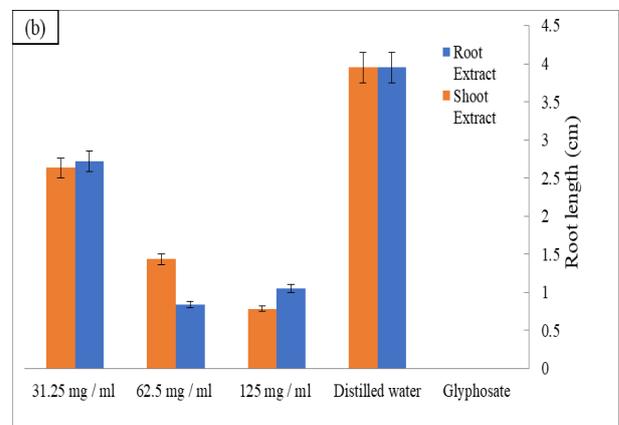
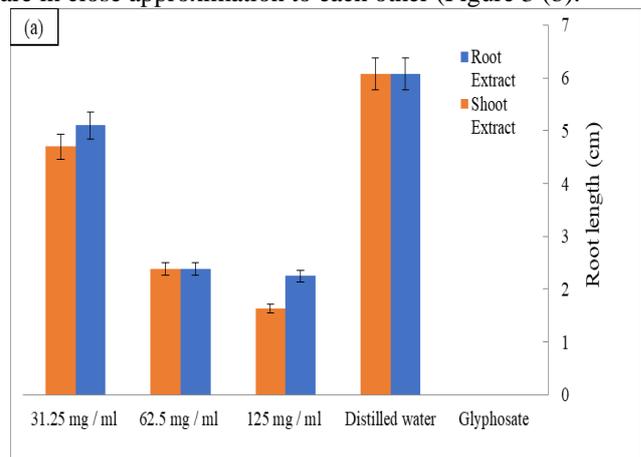


Figure III3. The Comparison of the effect of root and shoot water extracts of Imperata cylindrica on root length (cm) of (a) Cucumis sativus and (b) Lolium perenne

C. The comparison of the effect of root and shoot water extracts of Imperata cylindrica on shoot length of Cucumis sativus and Lolium perenne

The difference in terms of shoot length (cm) of Cucumis sativus by shoot and root extracts is described in Figure 4 (a). The positive and negative controls are shown at 0 cm and 4.5 cm as treated by glyphosate and distilled water, respectively. At the concentration of 125 mg/ml, shoot extract shows around 1.3 cm increases in shoot length as compared to the root extract.

There is a slight difference in the measurement of shoot length, that is, approximately 0.5 % between shoot extract and root extract at the concentration of 62.5 mg/ml. The shoot length significantly increased to about 5.5 cm in shoot extract as compared to root extract which shows shoot length of 4 cm. Overall, the shoot length graph shows an increased length of shoot extract as compared to root extract.

The effect of root and shoot extracts on shoot length of *Cucumis sativus* was estimated at three different concentrations. The positive and negative controls were measured by glyphosate and distilled water, respectively, within the range of 0 – 4.5 cm. At the concentration of 125 mg/ml, root water extract shows 1.75 cm of shoot length while shoot water extract has 0.75 cm shoot length. This shows that about a 1 cm increase in length is observed by root water extract. While opposite behavior has been observed at 62.5 mg/ml, which shows that shoot water extract has greater shoot length, that is, 2 cm, while root water extract has a lesser shoot length of 1.75 cm. Equal shoot lengths of 3.5 cm were shown by root and shoot water extracts at the concentration of 31.25 mg/ml (Figure 4 (b)).

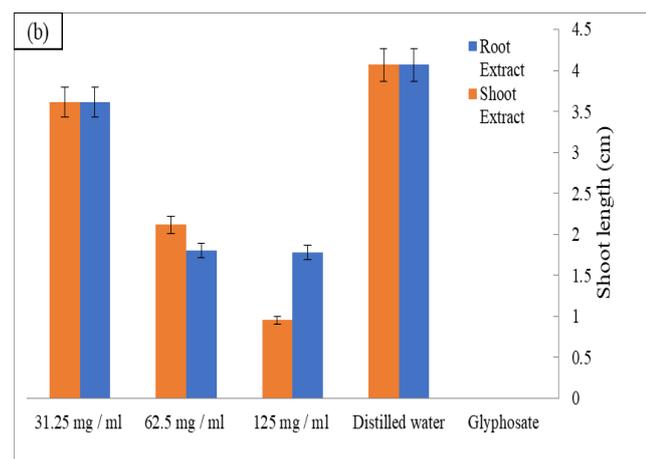
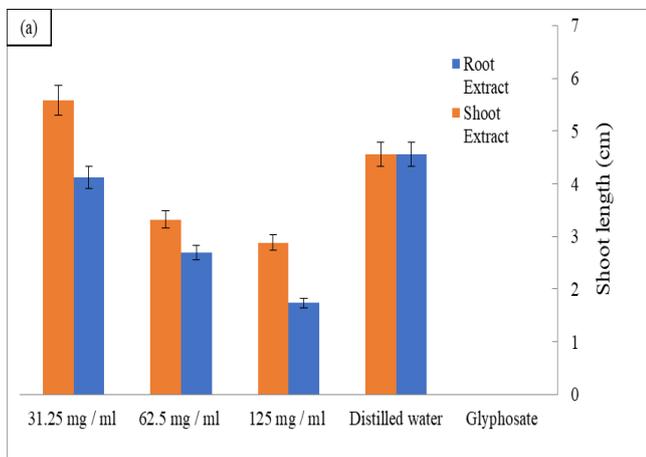


Figure III4. The comparison of the effect of root and shoot water extracts of *Imperata cylindrica* on shoot length (cm) of (a) *Cucumis sativus* and (b) *Lolium perenne*

D. The effect of root and shoot water extracts of *Imperata cylindrica* on the fresh weight of *Cucumis sativus* and *Lolium perenne*

The effect of *Imperata cylindrica* on weed *Cucumis sativus* in terms of fresh weight is shown in the comparison between shoot water extract and root water in Fig. 4.4 (a). 0 to 1.2 grams of fresh weight shows the range of positive control of

glyphosate and negative control of distilled water, respectively. At the concentration of 31.25 mg/ml, root water extract shows a fresh weight of 1.3 g and shoot water extract shows a fresh weight of 0.9 g. This shows that there is approximately a 0.4 g increase in fresh weight of root extract as compared to the shoot extract. Similarly, the concentration of 62.5 mg/ml shows the fresh weight of the root water extract of 1 g and shoot water extract of 0.65 g. So, an increase of 0.35g fresh weight was shown by root water extract. The same behavior was observed at the concentration of 125 mg/ml i.e. 0.4 g increase in fresh weight of root extract as compared to the shoot extract. The fresh weight of root extract has been observed to exceed (~1.3g) from negative control of water (~1.2g).

The effect of *Imperata cylindrica* extracts in terms of the fresh weight of weed *Lolium perenne* is shown in the comparison between shoot and root water extracts in Fig. 4 (b). 0 to 0.3 g of fresh weight shows the range of positive control of glyphosate and negative control of distilled water, respectively. At the concentration of 31.25 mg/ml, slight differences in fresh weight are observed in root and shoot water extracts. Similarly, the concentration of 62.5 mg/ml shows 0.05 g fresh weight in shoot water extract and 0.03 g in the root water extract. So, an increase of 0.02 g of fresh weight was shown by shoot water extract.

E. The effect of root and shoot water extracts of *Imperata cylindrica* on the dry weight of *Cucumis sativus* and *Lolium perenne*

Root and shoot extracts comparison in terms of dry weight (g) of *Cucumis sativus* is analyzed in Figure 5 (a). Positive and negative controls show 0 and 0.11 g dry weight, respectively. At three different concentrations, the root extract shows greater dry weight when compared to the shoot extract. All three different concentrations show ~0.02 g increase in dry weight in the root extract of *Imperata cylindrica* when compared to the shoot extract of the weed *Cucumis sativus*. Aqueous extract of *Imperata cylindrica* on weed *Cucumis sativus* shows that the % germination, root length, fresh weight, and dry weight exhibit greater root extract ratio as compared to shoot extract. Rusdy, Riadi [23] proposed that the aqueous extract of root and shoot parts of *Imperata cylindrica* exhibited an allelopathic effect on % germination, shoot, and root lengths, wet and dry weights. The highest allelopathic effect was recorded in shoot extract with >15 % concentration that completely inhibited shoot length and subsequently reduced the aerial growth of the tested plant [24].

The comparison of root and shoot extract in terms of dry weight (g) of *Lolium perenne* is analyzed in Figure 5 (b).

Positive and negative controls show the values of 0 and 0.009 g, respectively. At the concentration of 125 mg/ml, a minute difference of 0.0125 g increase in fresh weight of root water extract is shown as compared to the shoot extract [25]. At the concentration of 125 mg/ml, the dry weight of root water extract is 0.004 g while a dry weight of shoot water extract is 0.003g. This depicts that 0.001 g of dry weight is increased in the root water extract. 62.5 mg/ml

concentration shows that shoot water extract (~0.007 g) has greater dry weight as compared to root water extract (0.0045 g). Slight differences have been observed in the dry weight of root and shoot water extracts at the concentration level of 31.25 mg/ml. The highest allelopathic effect was reported in shoot extract with >15 % concentration that completely inhibited shoot length and subsequently reduced the aerial growth of the tested plant [23, 24, 26].

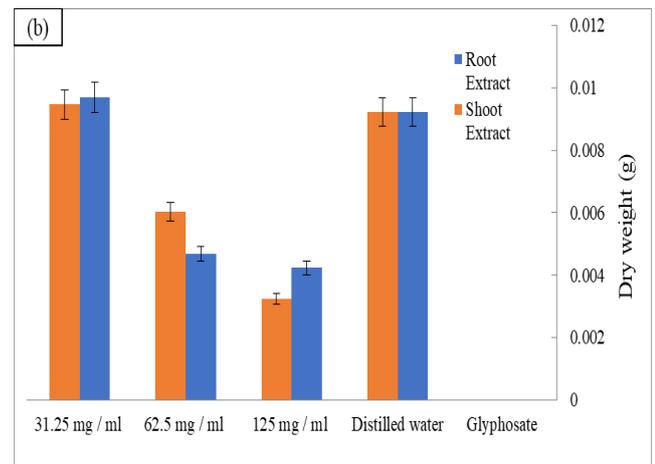
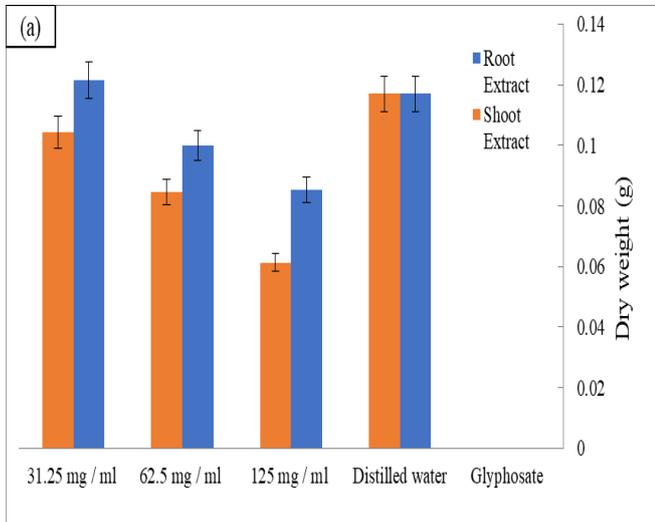


Figure. The comparison of the effect of root and shoot water extracts of *Imperata cylindrica* on the dry weight of (a) *Cucumis sativus* and (b) *Lolium perenne*.

From the present analysis and observations, it is obvious that although all plant part extracts have a strong allelopathic effect on seed germination and its growth of *Linum usitatissimum*, the highest inhibition was found by the shoot extract. Samad, Rahman [27] reported the higher inhibitory effect of the aqueous extracts of *Imperata cylindrica* leaf compared to other plant parts extracts on germination. Root and shoot extracts of all the applied concentrations significantly suppressed the germination of tested plants. Increasing the concentration of the extract increased the inhibitory potential. Results indicate that extracts of *Imperata cylindrica* may contain allelochemicals that may contribute to its invasiveness and extreme competitiveness.

IV. CONCLUSION

In this study, the allelopathic effect of the root and shoot aqueous extracts of *Imperata cylindrica* has been investigated on two weed species, that is, *Cucumis sativus* and *Lolium perenne*. The results of this study have revealed the allelopathic potential of the root and shoot's aqueous extracts of *Imperata cylindrica* in different concentrations as evidenced by the inhibition of percentage germination, root and shoot length, and fresh and dry weight of the tested weeds. The allelopathic effect of the tested extracts of *Imperata cylindrica* may be attributed to the presence of various phenolic compounds present in the extract. Further research is needed for the isolation and identification of the potential allelopathic compounds. We anticipate that the results of the present study would be beneficial for future research related to the allelopathic potential of *Imperata cylindrica* in terms of the isolation and identification of the active compounds.

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AUTHORS PROFILE



Mohamed Ali A. Salim is a PhD researcher in the Department of Technology & Natural Resources, Faculty of Applied Sciences & Technology, Universiti Tun Hussein Onn Malaysia. His research interests include isolation, purification and identification of allelopathic compounds from plants.



Dr. Alona Cuevas Linatoc is Associate Professor and Head of the Department of Technology & Natural Resources, Faculty of Applied Sciences & Technology, Universiti Tun Hussein Onn Malaysia. Her research interests include ecophysiology, plant physiology, medicinal plants, botany and plant diversity.



Dr. Suzi Salwah Jakin profile which contains their education details, their publications, research work, membership, achievements, with photo that will be maximum 200-400 words.



Dr. Shajarahunnur Jamil is Associate Professor in the Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia. Her research interests include natural products chemistry and organic synthesis.



Dr. Muhammad Sohail Latif is an MSc in Biochemistry and PhD in Biotechnology. His current research interests include the bioactive compounds, nanosynthesis of metallic nanoparticles and their application in pharmaceuticals, nutraceuticals and cosmaceuticals.