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Screening and Formulation of Tamarindus Indica and Citrus Limon Extract for Herbicidal Activity Against Parthenium Hysterophous

M. Nithya* [1], P. Sanjai [2] , P D Vivek [3], N Ajith[4], M Nirmal Kumar[5] and Baku Mansiben Devayatbhai[6]

Address: [1] M. Nithya, Assistant Professor, Department of Biotechnology, K. S. Rangasamy College of Technology, Tiruchengode, [2,3,4 and 5] B.Tech., Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode, Namakkal, Tamilnadu, [6] M.Tech., Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode, Namakkal, Tamilnadu

*Corresponding author: M. Nithya, Assistant Professor, Department of Biotechnology

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KEYWORDS

Allelopatheic effect, Tamarindus indica, seed germination, seedling growth, foliar spray, Parthenium hysterophorus, Herbicide

Abstract

The investigation demonstrated that the aqueous extracts from Tamarindus indica and Citrus limon displayed allelopathic effects on the growth of Parthenium hysterophorus across different concentrations (0%, 25%, 50%, and 75%). These effects resulted in a decrease in chlorophyll content and a reduction in plant height due to inhibited shoot and root elongation. All plants treated with the aqueous leaf extracts of Tamarindus indica and Citrus limon exhibited a significant decline in total chlorophyll content compared to the control treatments. Elevated concentrations of the aqueous seed and leaf extracts led to a more pronounced reduction in both root and shoot length. Moreover, the fresh and dry weight of the tested species significantly decreased after treatment with leaf aqueous extracts of Tamarindus indica and Citrus limon. This study highlights the allelopathic effects found in various plants, influencing neighboring vegetation through mechanisms such as volatilization, leaching, root exudation, decomposition, and herbicidal action.

INTRODUCTION

Controlling weeds is crucial in order to mitigate their negative impacts on crop production. Various weed control methods are available, including cultural practices, mechanical methods (such as hand weeding or mechanical cultivation), and chemical interventions (such as herbicide application). Integrated Weed Management (IWM) approaches that combine multiple control strategies are often recommended to achieve sustainable and effective weed control while minimizing the development of herbicide resistance.By employing appropriate weedcontrol measures, farmers can enhance crop productivity, improve crop quality, and reduce production costs, ultimately leading to better agricultural outcomes. (Hozyan et al 2010).

Parthenium hysterophorus, belonging to the Asteraceae family and commonly known as Parthenium or parthenium weed, holds considerable global significance and has been acknowledged in countries like Australia and India (Navie et al., 1996).This annual herb possesses a taproot system that can penetrate deeply into the soil, along with an upright shoot structure. In its early stages, Parthenium plants form a rosette of leaves close to the soil surface. Allelopathy, a form of plant interference, plays a substantial role in shaping ecosystem and agroecosystem dynamics. Natural herbicides offer a means to control weeds without relying on synthetic chemicals, thereby aiding in the promotion of sustainable agriculture practices. Utilizing natural herbicides can effectively target and eliminate weeds, leading to weed-free fields. This, in turn, contributes to increased agricultural efficiency and enhanced crop yields. By employing natural herbicides, farmers can tackle weed infestations while minimizing the use of synthetic chemicals. This approach aligns with the principles of environmentally friendly farming, as it reduces chemical inputs and their potential negative impacts on ecosystems and human health. The use of natural herbicides can thus be a valuable strategy in managing Parthenium and other weed species, helping to maintain healthy and productive agricultural systems.

The allelochemicals are alkaloids, flavonoids, terpenes, terpenoids, sorgoleone, phenolics and glycosides. The advantages of using plant extracts to control weeds as follows. They are used to reduce weeds and also to obtain the plants percentage of yield. They reduce the weeds organically without the use of chemicals. They are harmless to plants. They can reduce weeds as much as possible and increase the yield.

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MATERIALS AND METHODS

Preparation of extracts

Fresh leaf materials of Tamarindus indica and Citrus limon were gathered and cleaned by rinsing them with tap water first, followed by washing with sterilized water to remove contaminants. Subsequently, the leaves were blended in a 100-gram ratio, and the resulting leaf mixture was allowed to stand at a temperature of 20 °C for a period of 2 hours. After the incubation period, the mixture underwent filtration using Whatman No. 1 filter paper and a muslin sheet to remove any solid particles or impurities..The resulting filtrate obtained was considered as thestock solution, which was initially at a concentration of 100%. To obtain different concentrations, the stock solution was further diluted using sterilized water at 25% w/v solution 50% w/v solution and lastly, a 75% w/v solution. These percentages indicate the weight of the solute (in grams) per 100 milliliters of solution (w/v stands for weight/volume). Remember to properly measure and mix the components to ensure accurate and consistent results.It is important to note that the prepared extracts were used on the same day to ensure the freshness and effectiveness of the active compounds.

Petri Dish Bioassay Experiment

During the experiment, we tested various concentrations of leaf extracts to assess how different concentrations of the leaf extracts affected the overall development and establishment of Parthenium seedlings. By analyzing the results, we aimed to gain insights into the potential effects of the leaf extracts on this particular plant species.The experiment was conducted using Petri dishes of diameter 9cm. Each dish was lined with filter paper, and 10 Parthenium seeds were placed on the paper. The filter paper was then moisturized with 3 ml of various leaf extract concentrations, as the control distilled water was used . To ensure reliability every treatment was replicated three times.To maintain moisture levels, approximately two ml of the respective leaf extract or water was added to each dish every alternate day for a duration of 10 days. The Petri plates were kept at a temperature of 20℃±2 and were repeatedly tested to ensure proper moisture levels.For biochemical studies, the entire seedling was used to estimate total protein content by Lowry's et al. (1951). The cotyledons of the seedlings were used to determine the total chlorophyll content, following the method described by Arnon (1949). A separate control series was established employing solely water. During the initial seven days, two milliliters of the respective leaf extract or water was added daily to the Petri dishes to maintain appropriate moisture levels. Germination was documented when the radicle (root) emerged from the seed. The biophysical and biochemical traits of the seedlings were evaluated, with measurements of root length, shoot height, and fresh weight conducted on the 7th day. From each replication five seedlings were randomly chosen for measurement of growth and biomass parameters. In this experiment, the germination behavior of Parthenium was considered as a reliable indicator to evaluate the allelopathic action. The germination percentage of the Parthenium seeds in response to the various leaf extract concentrations was used to assess the allelopathic capability of the weed extracts.

Foliar spray bioassay

In this study, pots with a diameter and depth of 15 cm were employed, each filled with 600 g of sandy loam soil and planted with Parthenium seeds. Initially, ten seeds were sown in each pot, and one week after germination, the seedlings were thinned to maintain three uniform seedlings per pot. To investigate allelopathic effects, freshly prepared leaf extracts at concentrations of 25%, 50%, and 75% w/v were administered to the surface of Parthenium plants during the third and fourth weeks, using a hand sprayer. Subsequent sprays were conducted at 7-day intervals after the initial application. Control plots were treated with water instead of leaf extracts. Each treatment was duplicated three times to ensure dependable results. After four weeks from sowing, Parthenium seedlings were carefully uprooted and rinsed under running water to remove soil from the roots. Various biophysical and biochemical parameters of the seedlings, including root length, shoot height, fresh weight (FW), and dry weight (DW), were then measured.

Chlorophyll Estimation

The estimation of chlorophyll as described by Arnon (1949) was carried out. This method is commonly used for the determination of chlorophyll levels in plant samples.

RESULTS AND DISCUSSION

Petri dish Bioassay

The results from the experiment revealed a significant impact of the aqueous leaf extract of Tamarindus indica

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on the germination of Parthenium seeds.. Even at the lowest concentration examined (25%), the extract demonstrated considerable toxicity, leading to a significant 70% reduction in germination compared to the control group. This indicates that the extract has inhibitory effects on the germination process of Parthenium seeds. Further analysis and experimentation may be required for understanding the specific compounds or mechanisms involved in the observed toxicity.As the concentration of the extract increased, the germination rate was found to be decreased. The 50% and 75% of the extracts completely inhibited germination, resulting in a near-total reduction of approximately 100% when compared to the control group (Fig 1 and Table 1).

In the laboratory bioassay, it was noted that Tamarindus indica leaf extracts significantly impeded both the root and shoot growth of Parthenison. The 25% leaf extract concentration led to a substantial decrease of 73.27% in root length and 74.61% in shoot length. Higher concentrations of 50% and 75% completely halted both root and shoot growth, resulting in a 100% reduction in both parameters. Similarly, the lower concentration of 25% markedly reduced plant biomass by 79.65%. The higher concentrations of 50% and 75% demonstrated the most significant reduction in plant biomass, reaching 100% (Table 1). These findings emphasize that the aqueous leaf extracts of Tamarindus indica possess potent suppressing effects on the germination and growth of Parthenium, with higher concentrations exhibiting more pronounced impacts on seedling development and biomass accumulation.

Table 1: Effects of various Tamarindus indica aqueous leaf extract concentrations on the Germination percentage and seedling growth of Parthenium hysterophorus in Petridish bioassay

Treatment	Seed Germination	Root length (mm)	Shoot length (mm)	Plant fresh weight (mg)
Control(water)	100%	20.54	12.84	6.98
T1	30%	5.49	3.26	1.42
T2	0.00	0.00	0.00	0.00
T3	0.00	0.00	0.00	0.00

Where T1 = 25% aqueous leaf extracts concentration,T2 = 50% aqueous leaf extracts concentration, T3 = 75%aqueous leaf extracts concentration of Tamarindus indica.

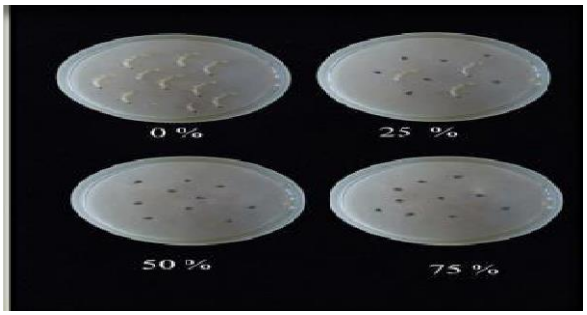

Figure 1. Effect of different concentration of aqueous extracts of Tamarindus indica leaves on the seed germination and early seedling growth of Parthenium hysterophorus

Results obtained from the laboratory bioassays revealed a significant inhibitory effect of the aqueous leaf extract of Citrus limon on both seed germination and the growth of Parthenium. Regarding seed germination, the extract demonstrated high toxicity, even at the lowest tested concentrations of 25% and 50%. At these concentrations, the extract caused substantial reductions in germination by 30% and 60%, respectively, compared to the control group. The inhibitory effect on germination exhibited a direct correlation with the increase in extract concentration. Moreover, the highest concentration of 75% completely inhibited germination,

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resulting in an approximately 100% reduction correlated to the control group (Fig 2 and Table 2). When treated with the aqueous leaf extract, the growth of root and shoot of Citrus limon also exhibited significant effects. Various concentrations of the extract had substantial impacts on the length of roots and shoots. The lower concentrations of 25% leaf extract significantly reduced root and shoot length by 56.46% and 45.09%, respectively. Concentrations of 50% leaf extract resulted in a more pronounced reduction, with root and shoot length decreasing by 68.20% and 58.72%, respectively. The highest concentration of 75% completely inhibited root and shoot growth, resulting in a reduction of 100% for both parameters.

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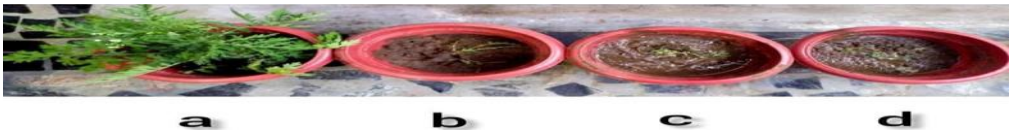
Table 2: Effect of different concentrations of aqueous leaf extract of Citrus limon on the early seedlinggrowth of Parthenium hysterophorus in laboratory bioassays

Treatment	Seed Germination	Root length (mm)	Shoot length (mm)	Plant fresh weight (mg)
Control(water)	100%	19.50	10.20	5.60
T4	60%	8.49	5.60	2.24
T5	40%	6.20	4.21	1.65
T6	0.00	0.00	0.00	0.00

Where T4 = 25% aqueous leaf extracts concentration, T5 = 50% aqueous leaf extracts concentration, T6 = 75%aqueous leaf extracts concentration of Citrus limon

Figure 2. Effect of different concentration of aqueous extracts of Citrus limon leaves on the seed germinationand early seedling growth of Parthenium hysterophorus Foliar spray Bioassay



a = control b= 25 % c= 50 % d= 75 %

Figure 3. Effect of different concentration of aqueous extracts of Tamarindus indica leaves on the growthof Parthenium hysterophorus

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The aqueous leaf extract of Tamarindus indica, especially at concentrations of 50% and 75%, demonstrated inhibitory effects on the root and shoot growth of Parthenium. As the concentration of the leaf extract increased, there was an intensified accumulation of chemicals, resulting in decreased plant growth, with the highest impact observed at concentrations of 50% and 75%, followed by the 25% concentration (Table 5). Polar compounds isolated from Tamarindus indica have been identified as potential contributors to its herbicidal activity against weeds. These findings emphasize the potential of Tamarindus indica leaf extract and its constituents as natural herbicides or agents for weed control. Further research is necessary to elucidate the specific mechanisms of action and explore their practical applications in weed management strategies. Moreover, the presence of sphingosine derivatives and a pyrrole derivative in Tamarindus indica leaves has been reported, which could also play a role in the plant's herbicidal activity (Sahai et al., 1999; Dabur et al., 2004).

Table 3: Effect of different concentrations of aqueous leaf extract of Tamarindus indica on the Germination percentage and seedling growth of Parthenium hysterophorus in Petridish bioassay

Treatments	Root Length (cm)	Shoot length (cm)	Root (mg/plant)		Shoot (mg/plant)	
			Fresh weight	Dry weight	Fresh weight	Dry weight
Control (water)	12.69	6.59	108.24	30.49	825.24	148.50
T1	8.80	3.94	80.75	13.00	700.00	119.49
T2	0.00	0.00	0.00	0.00	0.00	0.00
T3	0.00	0.00	0.00	0.00	0.00	0.00

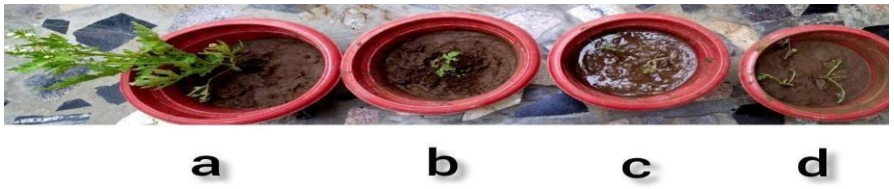

Figure 4. Effect of different concentration of aqueous extracts of Citrus limon leaves on the growth of Parthenium hysterophorus

The aqueous leaf extract of Citrus limon, especially at concentrations of 50% and 75%, demonstrated inhibitory effects on both root and shoot growth of Parthenium. The presence of the anolide compound in the leaf extract of Citrus limon may have indeed disrupted essential physiological processes responsible for plant growth,resulting in the observed effects. As the concentration of the leaf extract increased, the accumulation of chemicals intensified, leading to the suppression of plant growth, with the highest impact observed at concentrations of 50%and 75%, followed by the 25% concentration (Table 6).These findings suggest that the anolide compound present in the leaf extract of Citrus limon plays a role in inhibiting plant growth. Additional research is required to comprehend the precise mechanisms by which the anolide compound induces its inhibitory effects and to investigate potential applications of Citrus limon leaf extract as a natural herbicidal agent or for weed control. The polar compounds found in Citrus limon leaves may exhibit toxicity towards the germination and growth of Parthenium, thereby contributing to their inhibitory effects. These compounds are likely involved in disrupting the normal development and functioning of the weed, resulting in reduced root and shoot growth.

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Table 4: Effect of different concentrations of aqueous leaf extract of Citrus limon on the Germination percentage and seedling growth of Parthenium hysterophorus in foliar bioassay

Treatments	Root Length (cm)	Shoot length (cm)	Root (mg/plant)		Shoot (mg/plant)	
			Fresh weight	Dry Weight	Fresh weight	Dry Weight
Control (water)	11.49	5.59	100.24	26.49	795.24	135.50
T4	5.80	3.45	84.75	15.00	720.00	118.00
T5	4.25	2.00	50.26	10.00	625.00	101.23
T6	0.00	0.00	0.00	0.00	0.00	0.00

COMPARATIVE STUDIES

Figure 5: Effect of different concentration Tamarindus indica and Citrus limon on Partheniumhysteroporus in laboratory bioassay

In this chart, we can observe the effects of different treatments on Parthenium hysterophorus seed germination. The control treatment represents the use of water, while T1 represents the 25% aqueous leaf extracts concentration of Tamarindus indica, and T4 and T5 represent the 25% and 50% aqueous leaf extracts concentrations of Citrus limon, respectively.The results from the chart indicate that T1 (25% aqueous leaf extracts concentration of Tamarindus indica) had a greater impact on the length of root , shoot and fresh weight of the plant when compared to T4 and T5 (25% and 50% aqueous leaf extracts concentrations of Citrus limon, respectively). This suggests that Tamarindus indica was more effective than Citrus limon in inhibiting Partheniumhysterophorus seed germination .

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Estimation of Chlorophyll

Table 5: Allelopathic effects of aqueous leaf extracts of Tamarindus indica on chlorophyll biophysical parameters of Parthenium hysterophorus

Treatment	Chlorophyll a (mg/g)	Chlorophyll b(mg/g)	Total chlorophyll (a + b) (mg/g)
control	2.79	1.35	4.14
T1	2.05	1.01	3.06
T2	0.00	0.00	0.00
T3	0.00	0.00	0.00
T4	1.98	1.00	2.98
T5	1.25	0.90	2.15
T6	0.00	0.00	0.00

Where T1 = 25% aqueous leaf extracts concentration, T2 = 50% aqueous leaf extract concentration, T3 = 75%aqueous leaf extracts concentration of Tamarindus indica. T4 = 25% aqueous leaf extracts concentration, T5= 50% aqueous leaf extracts concentration, T6 = 75% aqueous leaf extracts concentration of Citrus limon.

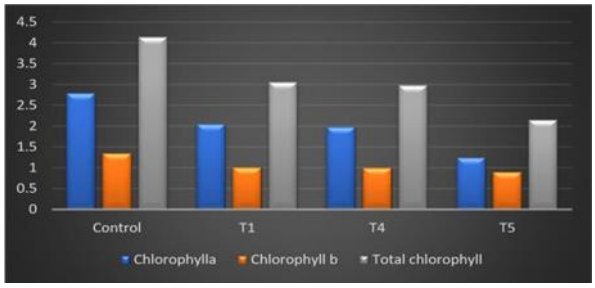

Figure 6: Total Chlorophyll in mg/gm of fresh weight in Parthenium hysteroporus

There was a notable reduction in chlorophyll content in all concentrations of Tamarindus indica and Citrus limon leaf extracts when compared to the control at the fourth week.. However, the reduction in chlorophyll content was more pronounced in the higher concentrations of the leaf extracts. These findings align with a previous study by Oyerinde et al. (2009) that demonstrated the inhibitory effect of allelopathic properties of Tithonia diversifolia on chlorophyll accumulation in young maize plants. Stupnicka-Rodzynkiewicz et al. (2006) conducted another study that similarly reported results concerning the influence of alleochemicals on chlorophyll content and photosynthesis in plants. The addition of aqueous leaf extracts from Tamarindus indica and Citrus limon significantly affects the germination efficiency and growth characteristics of Parthenium hysterophorus when compared to the control treatment. The observed reduction in chlorophyll content further indicates the potential allelopathic effects of these weed species on the target plant.

CONCLUSION

These findings underscore the potential of Tamarindus indica and Citrus limon as valuable sources of natural herbicides for the management of Parthenium , which is recognized as one of the most detrimental and environmentally harmful weeds globally. However, it is important to conduct further research to isolate and identify the precise active compounds responsible for the observed inhibitory effects in the leaf extracts of Tamarindus indica and Citrus limon. The identification of these compounds would facilitate the development of natural herbicides that can effectively control the growth of Parthenium while minimizing environmental pollution.By isolating and characterizing the specific active compounds, researchers can establish them as promising lead compounds for the synthesis of potent and environmentally friendly herbicides. This research direction holds great potential for sustainable weed management strategies that are both effective and ecologically sound. By identifying these compounds, researchers can gain a better understanding of their mechanisms of action and their potential as natural herbicides.Once the active compounds are isolated and

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identified, they can serve as lead compounds for the synthesis of natural herbicides that specifically target Parthenium weed while minimizing environmental pollution. This approach would provide a more targeted and sustainable method for weed control, reducing the reliance on synthetic chemical herbicides. Additional studies focusing on the mode of action, stability, and safety profile of these active compounds will be essential to ensure their potential as effective and sustainable herbicidal agents. Such advancements in natural herbicide development can contribute to the management of Parthenium weed and mitigate its negative impact on agricultural productivity and environmental sustainability.

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