Inhibition Potential of Leaf Extracts of Ornamental Shrubs *Plumeria rubra* and *Calotropis procera* on Germination and Growth of Selected Weeds - A Way to Reduce the Risk of Synthetic Herbicides

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Abstract

Allelopathy is a fascinating phenomenon where plants release chemical compounds that can affect the growth and development of neighboring plants. These allelochemicals can either encourage or hinder germination and growth, presenting an exciting opportunity for creating eco-friendly biological herbicides. Identifying plant materials that are readily available, cost-effective, and exhibit strong allelopathic properties could have significant environmental benefits as biological herbicides. Numerous studies have already demonstrated that certain plant-derived allelochemicals can effectively suppress weed growth. In this study, we investigated the allelopathic effects of extracts of two tropical ornamental shrubs, *Plumeria rubra* and *Calotropis procera*, through laboratory experiments.. The allelopathic effects of these extracts on seed germination and other germination traits were systematically analyzed. The findings showed that the combined application of 5% *Plumeria* + 10% *Calotropis* was highly effective for *Chenopodium album*, while the treatment of 10% *Plumeria* + 5% *Calotropis* was most effective for *Onobrychis sativa*, resulting in complete inhibition of germination. In contrast, the absence of extracts reduced stress effects and increased germination rates. The highest germination percentages were observed for *Onobrychis sativa* (55.00±5.010), *Rumex acetosella* (75.00±3.741), *Amaranthus retroflexus*

(100.00±5.849), *Tribulus terrestris* (21.67±2.415), and *Chenopodium album* (33.33±0.838). It was suggested that the combined application of 5% *Plumeria* + 10% *Calotropis* significantly inhibited the germination of weeds such as *Chenopodium album* and *Onobrychis sativa*. In conclusion, this study confirmed that the combined use of methanolic extracts holds promise as a potential raw material for developing pre-emergence herbicides targeting various weeds. This finding is significant as it offers scientific evidence that could support future research and development of eco-friendly herbicides.

Keywords: Calotropis, Plumeria, Inhibition, Weeds, Germination.

INTRODUCTION

One of the major causes of crop yield reduction is the invasion of weeds. Most studies attribute this reduction to various forms of competition between weeds and crops, often overlooking the allelopathic interactions between them. However, scientific findings since 1950 have indicated that allelopathic interactions between crops and weeds are partially responsible for yield losses in crops. While many weed species typically have inhibitory effects on crops, there are some that can actually enhance seed germination, growth, and overall yield in agricultural plants (Bao *et al*., 2019). Weeds influence crops by releasing phytotoxins through various means, including their seeds, decomposed residues, leachates, exudates, and volatile compounds. When sensitive plants are exposed to allelochemicals, their germination, growth, and development are affected (Li *et al*., 2020). In developed countries, where weed damage has been reduced to 5% through various management methods, large amounts of weed residues left in the soil after harvesting constitute a major source of soil phytotoxins (Chen et al., 2021). In developing countries, where weeds are not fully controlled, a portion of the crop yield is lost due to weed competition or their allelopathic effects. In such cases, understanding the type of interaction between weeds and crops can help in selecting appropriate weed management strategies (Shen et al., 2017).

Allelopathy offers an alternative strategy for weed management. In the future, using this strategy could reduce the application of traditional herbicides in soil, replacing them with biological herbicides (Bao *et al*., 2015). The search for and development of new herbicides involve isolating, identifying, and synthesizing new compounds from plants with allelopathic potential, a

process that begins with recognizing the allelopathic capacity of plants (Mushtaq *et al*., 2024). Several plants with allelopathic activity have been identified in this regard (Xie et al., 2024).

To determine the allelopathic activity of plants, various bioassays such as seed germination, root elongation, and seedling growth are employed (Fazeli-Nasab *et al*., 2023). Seed germination does not exhibit consistent sensitivity across all experiments (Moradi *et al*., 2023). Root elongation is more sensitive and has been widely used in various laboratories. Seedling growth is highly sensitive, as it involves numerous physiological processes that may be altered by allelochemicals (Rawat. *et al*., 2017). Allelochemicals influence multiple factors, such as mineral uptake, water relations, chlorophyll content, respiration, and photosynthesis. Seedling bioassays are used to quantitatively observe morphological effects (Mlombo *et al*., 2024).

To date, many commercial herbicides derived from plants or plant extracts have been successfully developed around the world. Notably, in 1996, Cook isolated a compound known as strigol from the root exudates of cotton. This compound has proven effective in promoting the germination of Striga asiatica seeds (Humphrey and Beale, 2006). Strigol treatment triggered "suicidal germination" in *Striga asiatica*, *Orobanche coerulescens*, and other parasitic weeds, even in the absence of a host. Additionally, Trigonoxazonan, secreted by the root system of *Trigonella foenum-graecum*, effectively inhibited the germination of *Orobanche crenata* seeds, a parasitic weed that invades legume crop fields (Bao *et al*., 2019).

In summary, plant-derived allelochemicals hold significant research value and offer broad applications. Therefore, it is imperative to investigate more plant materials with extensive availability, low cost, and significant allelopathic effects as potential herbicides. Calotropis procera is a shrub belonging to the Apocynaceae family. Calotropis procera is a sun-loving plant and is native to Africa, the Arabian Peninsula, and South and Southeast Asia (Rahman and Wilcock, 1991). Calotropis plays an effective role in controlling tall weeds, shrubs, and especially grasses, and is also a suitable bioindicator for monitoring pollution in urban and suburban areas. In addition, Calotropis has an allelopathic effect on the germination and rooting of other plants. Allelochemicals present in the leaves and roots of Calotropis prevent the germination and growth of millet seeds (Samreen *et al*., 2009).

Plumeria belongs to the Apocynaceae family and is also known as frangipani and Champa. It is a small tree growing to a height of about 7-8 m (Stephen, 2008; Sunita, 2016) and is considered

as an excellent ornamental plant and is known for its attractive appearance along with fragrant flowers (Kapoor and Sharga, 1993). According to our research, there is no report of allelopathic effect of this plant extract on weed seed germination. Muthusamy (2014) reported that Plumeria flowers have mosquito (Aedes aegypti) repellent potential. In Iran, allelopathy research began some time ago, and several studies have been conducted in this regard. The aim of this experiment was to evaluate the allelopathic effects of methanolic extracts from the aerial parts of *Plumeria* and *Calotropis* on the germination and growth of selected weeds (*Onobrychis sativa*, *Rumex acetosella*, *Amaranthus retroflexus*, *Tribulus terrestris*, and *Chenopodium album*).

MATERIALS AND METHODS

Plant Materials

The aerial parts of *Plumeria* and *Calotropis* were collected during the early flowering stage from the research collection of the Faculty of Agriculture, Hormozgan University. The materials were placed separately in cloth bags and transported to the laboratory. Leaves were separated from other plant parts, placed in paper envelopes, and dried in an oven at 75°C for 48 hours.

Preparation of Extracts

To prepare aqueous extracts, 10 grams of dried powder of *Plumeria* and *Calotropis* were mixed with 100 mL of distilled water and allowed to stand for 48 hours. The samples were then ground using an electric grinder and passed through a 40-mesh sieve. The filtered extract was diluted to concentrations of 10% (10 mL extract and 90 mL distilled water) and 5% (5 mL extract and 95 mL distilled water) (Ahn, and Chung, 2000). The resulting solution was filtered twice using Whatman No. 42 filter paper (Chon *et al*., 2002).

Field Trial

Seeds of *Onobrychis sativa*, *Rumex acetosella*, *Amaranthus retroflexus*, *Tribulus terrestris*, and *Chenopodium album* were counted in groups of 50. They were sterilized with a 10% bleach

solution (containing 5.25% sodium hypochlorite) for 3 minutes and then rinsed five times with distilled water [15]. Four replicates were used for each treatment. The seeds were placed in culture dishes, and 5 mL of the prepared solution was added to each Petri dish. To prevent microbial contamination and evaporation, the dishes were covered with transparent film (Chadho *et al*., 1995). Germination was recorded 24 hours after treatment and subsequently monitored every 12 hours.

Seed Germination Assays

The evaluated traits in this experiment included: germination percentage, mean germination time, germination index, germination rate, root length, and shoot length (Aflakpui *et al*., 1998).

Qualitative Traits Measured

Germination Percentage: Number of germinated seeds/Total number of seeds × 100

Germination Speed: The germination speed was determined using Maguire's (Maguire, 1962). method with the following formula:

$$Rs = \sum_{i=1}^{n} \frac{Si}{Di}$$

Rs: Germination speed (number of seeds per day)

Si: Number of germinated seeds in each count

Di: Number of days until the nth count

Statistical Analysis

Statistical analyses commenced with descriptive statistics to evaluate the initial quality of the collected data. This step aimed to simplify and condense large datasets for easier interpretation. Following this, more advanced analyses were conducted. The Duncan's Multiple Range Test (DMRT) was performed

using SPSS software (version 26; IBM Corp., Armonk, NY, USA) to calculate mean squares and experimental errors, helping to identify differences between datasets. The DMRT was applied at a significance level of $p \leq 0.05$ to detect differences between means.Additionally, Pearson's correlation coefficient was computed using SPSS (version 26; IBM Corp., Armonk, NY, USA) to assess the relationships between various parameter pairs. All calculations, including normality of data distribution and simple correlation coefficients, were carried out using SPSS version 26. A causality diagram was created using AMOS software (version 24).

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RESULTS AND DISCUSSION

Analysis of Variance Results

According to the analysis of variance results, all traits were significantly affected by the factors of plant species, extract concentration, and their interaction. However, the germination percentage (GP) was not significantly influenced by the extract concentration factor. The remaining traits showed significant differences at the 1% significance level ($p \leq 0.01$) (Table 1).

Table 1. Mean squares of the effects of plant species and extract concentration on the indices (GP, MGT, GS, GI) of selected weeds.

So V	df	MS			
		GP	MGT	GS	GI
Plants	4	9395.83**	113.91**	574.93*	47.27**
Extracts	4	4839.16**	8.91^{ns}	1176.18**	56.63**
Plants * Extracts	16	476.66**	24.92**	500.75**	15.08**
Error	66	68.00	5.90	135.45	0.090
Cv (%)	-	8.97	10.58	8.14	6.49

Continuation of Table 1. Mean squares of the effects of plant species and extract concentration on the indices (MDGP, SL, RL) of selected weeds.

So V	df	MS		
		MDGP	SL	RL
Plants	4	332.34**	28.65**	17.53**
Extracts	4	327.77**	4.19**	10.49**
Plants* Extracts	16	111.06**	3.45**	3.36**
Error	66	1.629	0.641	0.754
Cv (%)	-	1.68	2.98	6.98

The comparison of means for the plant species factor revealed that the highest values for all traits, except for MGT, were observed in *Amaranthus retroflexus*. The lowest values for GP,

GS, GI, and MDGP were observed in *Chenopodium album*, while for MGT, GI, and RL, the lowest values were found in *Rumex acetosella* (Table 2).

Table 2. Comparison of the simple main effect of selected weeds on germination indices.

Species	GP	MGT	GS	GI	MDGP	SL	RL
Onobrychis sativa	13.33^{bc}	2.54^{c}	16.32^{ab}	1.62^{b}	2.55^{b}	0.57^{c}	0.31^{c}
Rumex acetosella	19^{b}	9.71^{a}	7.81^{c}	0.38^{c}	1.49^{c}	0.20^{c}	0.00^{c}
Amaranthus retroflexus	69.66^{a}	6.29^{b}	22.07^{a}	4.50^{a}	12.30^{a}	3.40^{a}	2.43^{a}
Tribulus terrestris	15.33^{bc}	3.98^{bc}	15.23^{ab}	0.44^{c}	2.44^{b}	0.00^{c}	0.00^{c}
Chenopodium album	9.33^{c}	4.50^{bc}	7.43^{c}	0.38^{c}	1.01^{c}	1.29^{b}	1.50^{b}

The comparison of the mean effect of extract concentration showed that the highest germination trait values were observed in the control treatment (distilled water), while the lowest values were recorded in the 10% combined treatment (Table 3). This indicates that applying treatments at different concentrations led to a decrease in the germination traits, and as the concentration increased, the reduction in trait values became more pronounced.

Table 3. Comparison of the mean effect of extract concentration on germination indices.

Extract treatment	GP	MGT	GS	GI	MDGP	SL	RL
5% Plumeria + 5% Calotropis	21.33^{b}	6.63^{a}	11.83^{b}	0.85^{b}	1.87^{bc}	1.28^{ab}	1.62^{a}
5% Plumeria + 10% Calotropis	16.66^{bc}	5.3724.^{a}	9.53^{b}	0.62^{b}	1.72^{bc}	0.69^{bc}	0.36^{b}
10% Plumeria + 5% Calotropis	18.66^{bc}	5.39^{a}	13.86^{b}	0.64^{b}	2.68^{b}	0.44^{c}	0.22^{b}
10% Plumeria + 10% Calotropis	13^{c}	4.51^{a}	5.12^{b}	0.28^{c}	1.25^{c}	1.27^{ab}	0.15^{b}
Control treatment	57^{a}	5.12^{a}	28.50^{a}	4.92^{a}	12.27^{a}	1.77^{a}	1.88^{a}

The results of the mean comparison of the interactions of factors on traits such as germination percentage (Table 4), mean germination time (Table 5), germination speed (Table 6), germination index (Table 7), average daily germination percentage (Table 8), shoot length (Table 9), and root length (Table 10) showed that the combined treatment of 5% Plumeria + 10% Calotropis for *Chenopodium album* and 10% Plumeria + 5% Calotropis for *Onobrychis sativa* was highly effective. In contrast, the absence of extract application reduced the stress effects, leading to an increase in germination (Table 4). The highest germination percentages were observed in *Onobrychis sativa* (55.00±5.010), *Rumex acetosella* (75.00±3.741), *Amaranthus retroflexus* (100.00±5.849), *Tribulus terrestris* (41.67±2.415), and *Chenopodium album* (33.33±0.838).

Table 4. Interaction effect of plant extracts and concentration on the germination percentage of selected weeds.

Extract treatment	*Onobrychis sativa*	*Rumex acetosella*	*Amaranthus retroflexus*	*Tribulus terrestris*	*Chenopodium album*
5% Plumeria + 5% Calotropis	8.33±1.607^{h-k}	8.33±0.007^{h-k}	81.67±3.218^{b}	3.33±0.003^{jk}	5.00±0.002^{ijk}
5% Plumeria + 10% Calotropis	1.67±0.006^{jk}	5.00±0.004^{ijk}	61.67±1.231c^{d}	15.00±0.005^{h-k}	0.00±0.000^{k}
10% Plumeria + 5% Calotropis	0.00±0.000^{k}	5.00±0.003^{ijk}	61.67±1.221c^{d}	20.00±1.257^{ghi}	6.67±0.016^{h-k}
10% Plumeria + 10% Calotropis	1.67±0.006^{jk}	1.67±0.001^{jk}	43.33±0.052^{ef}	16.67±1.114^{hij}	1.67±0.001^{jk}
Control treatment	55.00±5.010^{de}	75.00±3.741^{b}c	100.00±5.849^{a}	41.67±2.415^{gh}	33.33±0.838^{fg}

The application of 5% Plumeria + 5% Calotropis also resulted in an increase in the mean germination time. The mean germination time trait in the plants *Onobrychis sativa* (4.33±0.033), *Rumex acetosella* (12.83±0.441), *Amaranthus retroflexus* (8.86±0.514), *Tribulus terrestris* (6.89±0.484), and *Chenopodium album* (7.83±0.941) showed the highest values (Table 5).

Table 5. Interaction effect of plant extracts and concentration on the mean germination time of selected weeds.

Extract treatment	*Onobrychis sativa*	*Rumex acetosella*	*Amaranthus retroflexus*	*Tribulus terrestris*	*Chenopodium album*
5% Plumeria + 5% Calotropis	4.33±0.167^{d-h}	12.83±0.441^{a}	6.49±0.525^{c-g}	1.67±0.006^{h}	7.83±0.941^{b-e}
5% Plumeria + 10% Calotropis	2.00±0.002^{gh}	10.67±0.333^{abc}	8.86±0.514^{a-d}	5.36±0.073^{d-h}	0.00±0.000^{i}
10% Plumeria + 5% Calotropis	0.00±0.000^{i}	11.33±0.333^{ab}	6.99±0.097^{b-f}	2.33±0.333^{fgh}	6.33±0.333^{c-h}
10% Plumeria + 10% Calotropis	4.33±0.033^{d-h}	3.67±0.067^{e-h}	7.23±0.384^{b-e}	3.67±0.667^{e-h}	3.67±0.066^{e-h}
Control treatment	2.07±0.032^{gh}	10.09±0.164^{abc}	1.88±0.044	6.89±0.484^{b-f}	4.70±0.252^{d-h}

The absence of extract application resulted in a reduction in the stress effects and an increase in the germination speed (Table 6). The highest germination speed was observed in *Onobrychis sativa* (50.51±2.887), *Rumex acetosella* (9.92±0.164), *Amaranthus retroflexus* (53.80±0.871), *Tribulus terrestris* (38.89±3.098), and *Chenopodium album* (21.39±0.167).

Table 6. Interaction effect of plant extracts and concentration on the germination speed of selected weeds.

Extract treatment	*Onobrychis sativa*	*Rumex acetosella*	*Amaranthus retroflexus*	*Tribulus terrestris*	*Chenopodium album*
5% Plumeria + 5% Calotropis	23.15±0.926^{bc}	7.88±0.610^{c}	15.79±0.343^{c}	6.67±1.154^{c}	5.70±0.268^{c}
5% Plumeria + 10% Calotropis	5.40±0.154^{c}	9.39±0.303^{c}	12.25±0.189^{c}	20.65±0.987^{bc}	0.00±0.000^{c}
10% Plumeria + 5% Calotropis	0.00±0.000^{c}	8.84±0.253^{c}	14.58±0.304^{c}	38.89±3.098^{ab}	7.04±0.516^{c}
10% Plumeria + 10% Calotropis	2.56±0.056^{c}	3.03±0.030^{c}	13.96±0.439^{c}	3.03±0.030^{c}	3.03±0.030^{c}
Control treatment	50.51±2.887^{a}	9.92±0.164^{c}	53.80±0.871^{a}	6.92±0.148^{c}	21.39±0.167^{bc}

The highest value for the germination index was observed in *Onobrychis sativa* (7.53±0.312), *Rumex acetosella* (1.60±0.030), *Amaranthus retroflexus* (13.67±0.167), *Tribulus terrestris* (1.16±0.012), and *Chenopodium album* (1.65±0.022) (Table 7).

Table 7. Interaction effect of plant extracts and concentration on the germination index of selected weeds.

Extract treatment	*Onobrychis sativa*	*Rumex acetosella*	*Amaranthus retroflexus*	*Tribulus terrestris*	*Chenopodium album*
5% Plumeria + 5% Calotropis	0.51±0.013^{gh}	0.13±0.018^{h}	3.48±0.296^{c}	0.14±0.001^{h}	0.08±0.001^{h}
5% Plumeria + 10% Calotropis	0.06±0.001^{h}	0.09±0.002^{h}	1.84±0.162^{de}	1.16±0.012^{fe}	0.00±0.000^{h}
10% Plumeria + 5% Calotropis	0.00±0.000^{h}	0.09±0.002^{h}	2.27±0.142^{d}	0.72±0.041^{fg}	0.15±0.005^{h}
10% Plumeria + 10% Calotropis	0.03±0.001^{h}	0.03±0.001^{h}	1.32±0.013^{ef}	0.03±0.01^{h}	0.03±0.001^{h}
Control treatment	7.53±0.312^{b}	1.60±0.030^{ef}	13.67±0.167^{a}	0.17±0.001^{h}	1.65±0.022^{ef}

The highest value for the average daily germination percentage was observed in *Onobrychis sativa* (11.03±0.818), *Rumex acetosella* (5.79±0.405), *Amaranthus retroflexus* (38.89±0.556), *Tribulus terrestris* (6.11±0.568), and *Chenopodium album* (3.90±0.151) (Table 8).

Table 8. Interaction effect of plant extracts and concentration on the daily mean germination percentage of selected weeds.

Extract treatment	*Onobrychis sativa*	*Rumex acetosella*	*Amaranthus retroflexus*	*Tribulus terrestris*	*Chenopodium album*
5% Plumeria + 5% Calotropis	1.37±0.060^{g}	0.63±0.087^{g}	6.48±0.321^{c}	0.52±0.001^{g}	0.39±0.002^{g}
5% Plumeria + 10% Calotropis	0.28±0.002^{g}	0.47±0.015^{g}	5.54±0.202^{cd}	2.34±0.151^{efg}	0.00±0.000^{g}
10% Plumeria + 5% Calotropis	0.00±0.000^{g}	0.44±0.013^{g}	6.25±0.089^{cd}	6.11±0.568^{cd}	0.62±0.012^{g}
10% Plumeria + 10% Calotropis	0.12±0.001^{g}	0.15±0.012^{g}	4.36±0.214^{cde}	1.52±0.051^{g}	0.15±0.001^{g}
Control treatment	11.03±0.818^{b}	5.79±0.405^{cd}	38.89±0.556^{a}	1.77±0.019^{fg}	3.90±0.151^{def}

The results of the comparison of the interaction effects on the hypocotyl length trait (Table 9) showed that the combined application was fully effective for *Tribulus terrestris*, with no significant effect observed. The highest values for the average daily germination percentage were found in *Onobrychis sativa* (2.87±0.145), *Rumex acetosella* (1.00±0.001), *Amaranthus retroflexus* (5.11±0.048), and *Chenopodium album* (3.91±0.029) (Table 9).

Table 9. Interaction effect of plant extracts and concentration on the shoot length of selected weeds.

Extract treatment	*Onobrychis sativa*	*Rumex acetosella*	*Amaranthus retroflexus*	*Tribulus terrestris*	*Chenopodium album*
5% Plumeria + 5% Calotropis	0.00±0.000^{e}	0.00±0.000^{e}	5.11±0.048^{a}	0.00±0.000^{e}	1.30±0.061^{de}
5% Plumeria + 10% Calotropis	0.00±0.000^{e}	0.00±0.000^{e}	3.46±0.043^{bc}	0.00±0.000^{e}	0.00±0.000^{e}
10% Plumeria + 5% Calotropis	0.00±0.000^{e}	0.00±0.000^{e}	2.23±0.028^{cd}	0.00±0.000^{e}	0.00±0.000^{e}
10% Plumeria + 10% Calotropis	0.00±0.000^{e}	1.00±0.001^{de}	4.10±0.051^{ab}	0.00±0.000^{e}	1.27±0.001^{de}
Control treatment	2.87±0.0.145^{bc}	0.00±0.000^{e}	2.11±0.031^{cd}	0.00±0.000^{e}	3.91±0.029^{ab}

The results of the comparison of the interaction effects on the root length trait (Table 10) showed that the combined application was fully effective for *Rumex acetosella* and *Tribulus terrestris*, with no significant effect observed. The highest values for the average daily germination percentage were found in *Onobrychis sativa* (1.57±0.033), *Amaranthus retroflexus* (4.77±0.417), and *Chenopodium album* (3.67±0.333) (Table 10).

Table 10. Interaction effect of plant extracts and concentration on the root length of selected weeds.

Extract treatment	***Onobrychis sativa***	***Rumex acetosella***	***Amaranthus retroflexus***	***Tribulus terrestris***	***Chenopodium album***
5% Plumeria + 5% Calotropis	0.00 ± 0.000^{e}	0.00 ± 0.000^{e}	4.47 ± 0.256^{ab}	0.00 ± 0.000^{e}	3.67 ± 0.333^{ab}
5% Plumeria + 10% Calotropis	0.00 ± 0.000^{e}	0.00 ± 0.000^{e}	1.83 ± 0.033^{cd}	0.00 ± 0.000^{e}	0.00 ± 0.000^{e}
10% Plumeria + 5% Calotropis	0.00 ± 0.000^{e}	0.00 ± 0.000^{e}	1.11 ± 0.030^{de}	0.00 ± 0.000	0.00 ± 0.000^{e}
10% Plumeria + 10% Calotropis	0.00 ± 0.000^{e}	0.00 ± 0.000^{e}	0.00 ± 0.000^{e}	0.00 ± 0.000^{e}	0.75 ± 0.007^{de}
Control treatment	1.57 ± 0.033^{de}	0.00 ± 0.000^{e}	4.77 ± 0.417^{a}	0.00 ± 0.000^{e}	3.10 ± 0.014^{bc}

Correlation Coefficients Results

The Pearson correlation coefficients between the studied traits are presented in Fig. 1-5. The results showed that only the MGT trait did not have a significant correlation with the other traits, while the remaining traits showed a significant positive correlation at the 1% level for *Onobrychis sativa* (Fig.1).

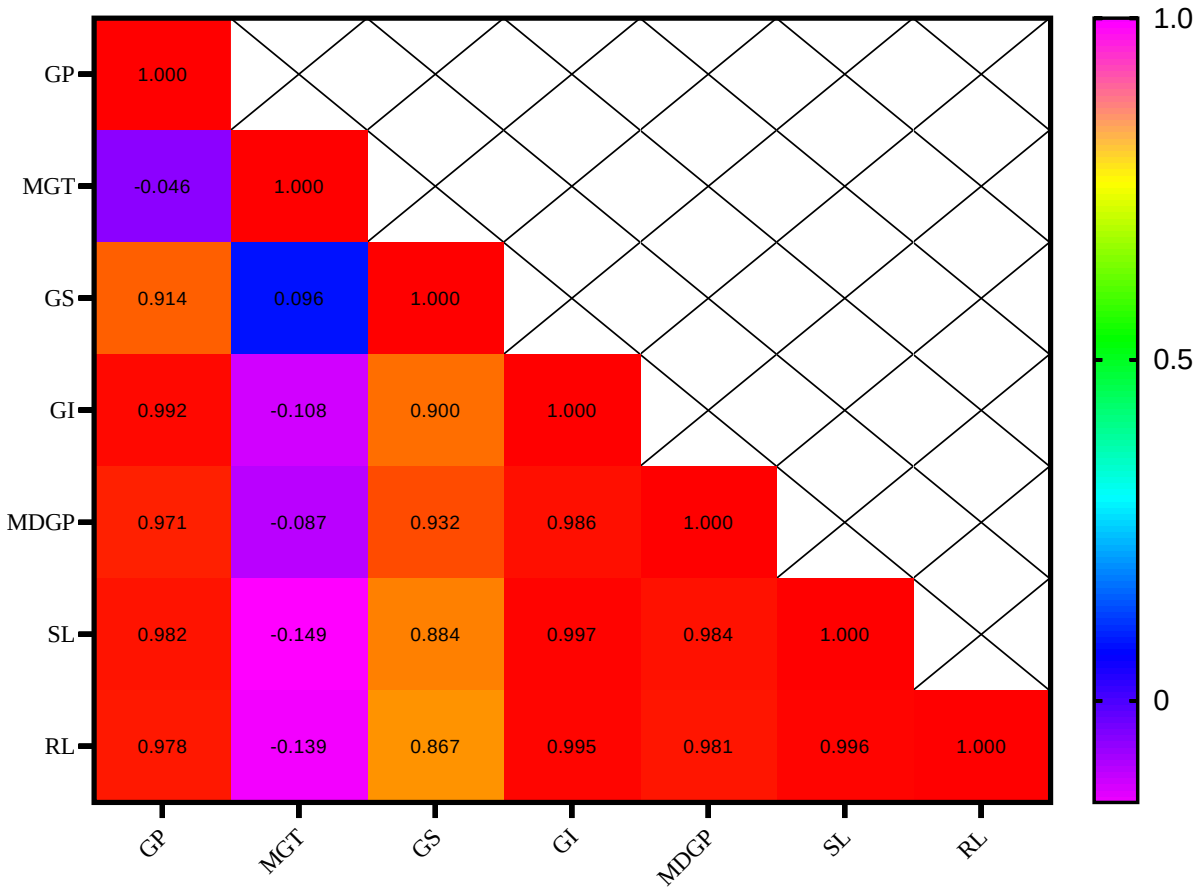

Fig. 1. Heatmap of the interrelationships of variables in the correlation coefficient for the traits studied in the plant *Onobrychis sativa*.

The results showed that the GP trait had the highest positive and significant correlations with the MDGP (0.997**), GI (0.989**), and GP (1.00**) traits. Additionally, the GI trait had the highest positive and significant correlation with the MDGP (0.986**) trait for *Rumex acetosella* (Figure 2).

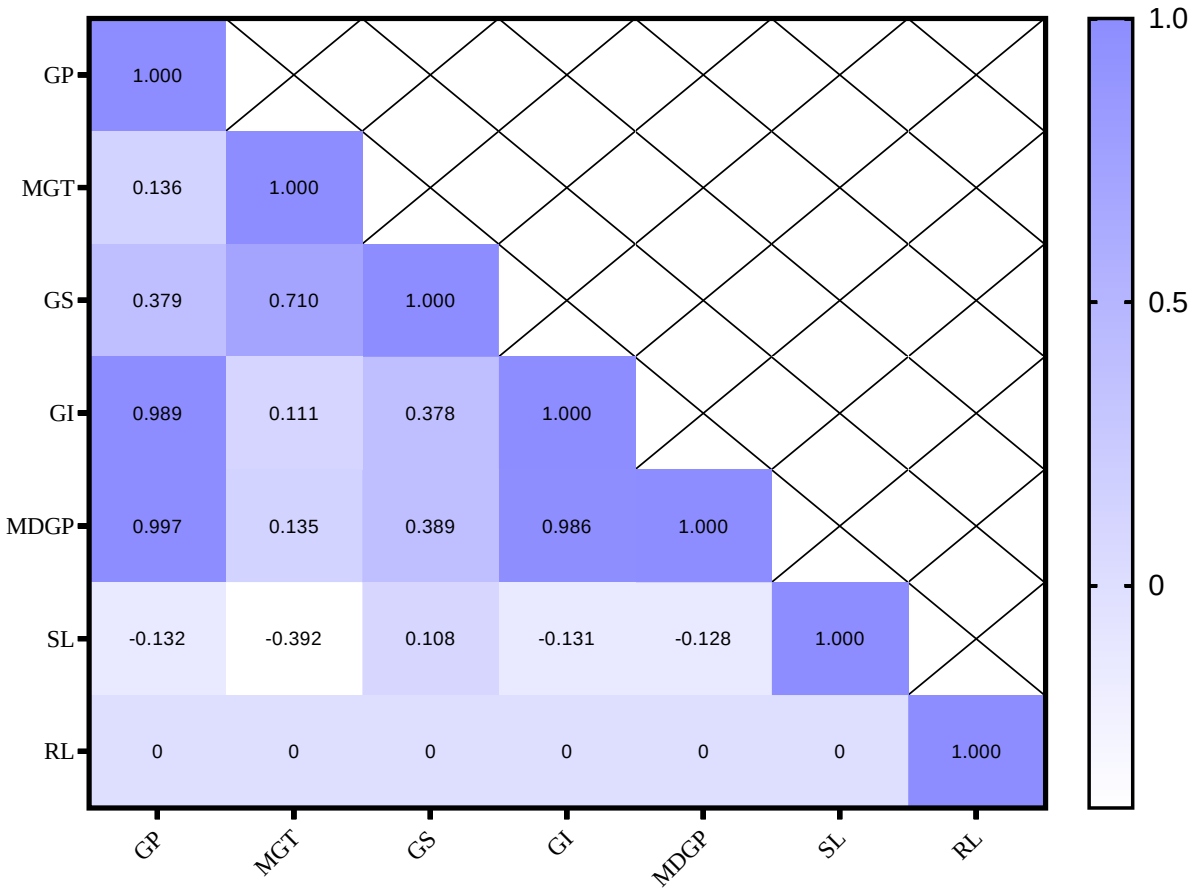

Fig. 2. Heatmap of the interrelationships of variables in the correlation coefficient for the traits studied in the plant *Rumex acetosella*.

The results showed that the GP trait had the highest positive and significant correlations with RL (0.877**), MDGP (0.772**), GI (0.825**), GS (0.755**), and GP (1.000**). The GS trait had the highest positive and significant correlations with RL (0.642**), MDGP (0.997**), and GI (0.992**). Additionally, the GI trait showed the highest positive and significant correlations with RL (0.711**) and MDGP (0.993**), while the MDGP trait had the highest positive and significant correlation with RL (0.643**) for *Amaranthus retroflexus* (Fig. 3).

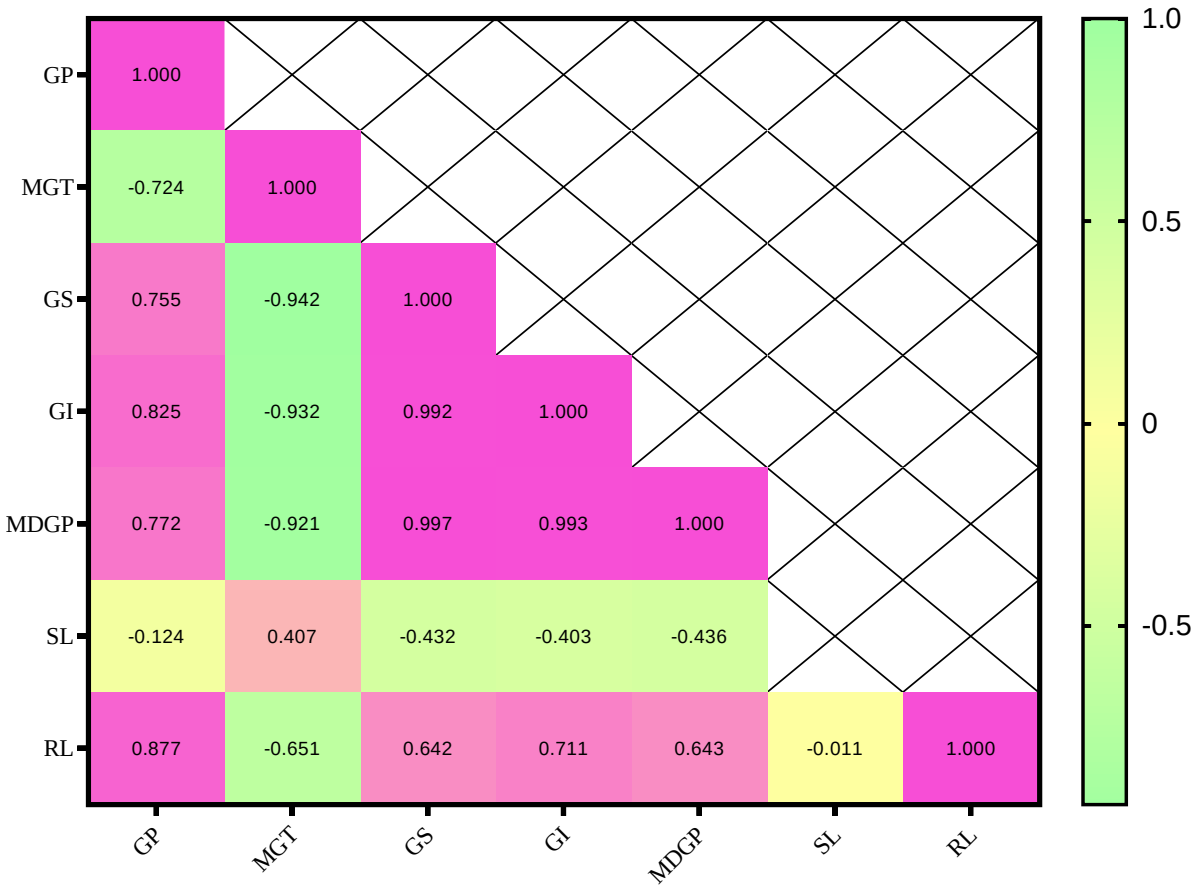

Fig. 3. Heatmap of the interrelationships of variables in the correlation coefficient for the traits studied in the plant *Amaranthus retroflexus*.

The results showed that the GP trait had the highest positive and significant correlations with MDGP (0.470*) and MGT (0.837**). The GS trait had the highest positive and significant correlations with MDGP (0.770**) and GI (0.852**). Additionally, the MDGP trait showed the highest positive and significant correlation with GI (0.566**) for *Tribulus terrestris* (Fig. 4).

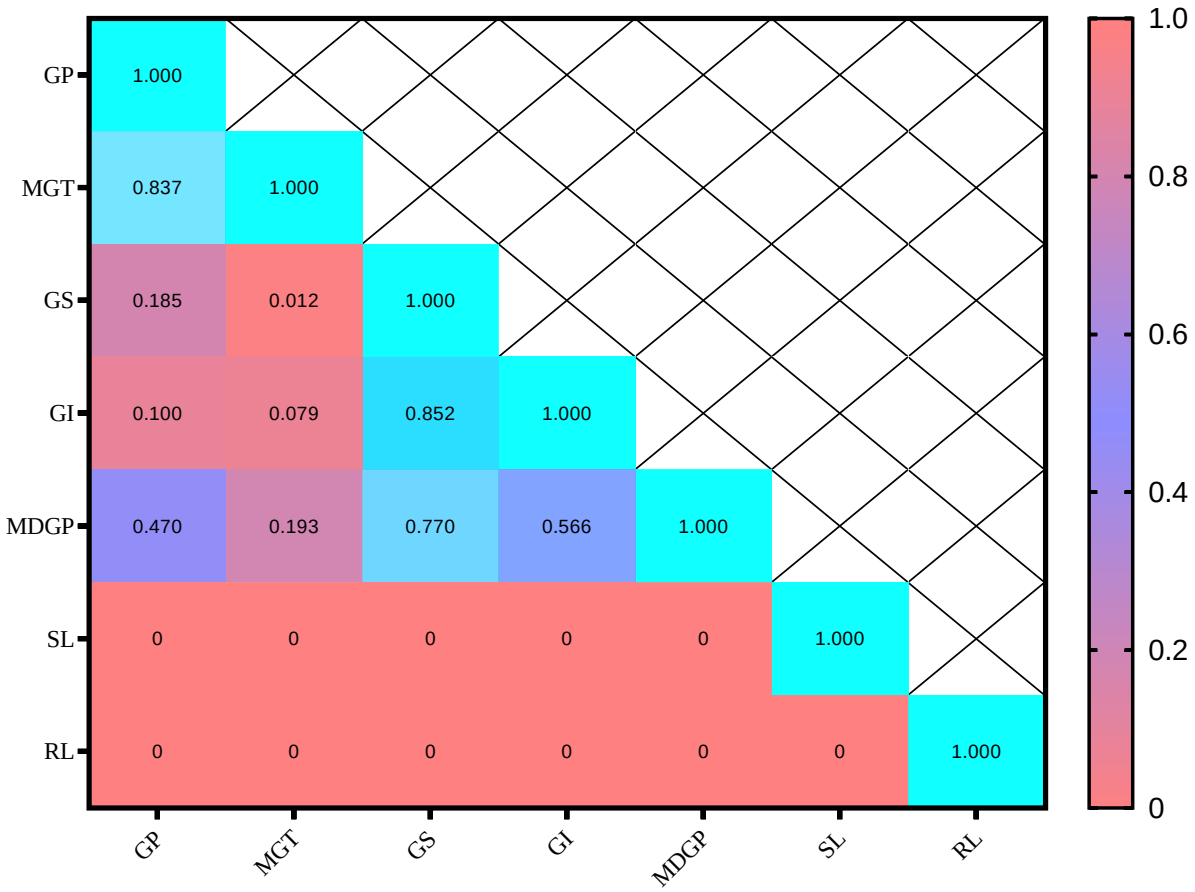

Fig. 4. Heatmap of the interrelationships of variables in the correlation coefficient for the traits studied in the plant *Tribulus terrestris.*

The results showed that the GP trait had the highest positive and significant correlations with MDGP (0.939^{**}), GI (0.950^{**}), and GS (0.875^{**}). The highest positive and significant correlation for the GS trait was observed with SL (0.792^{**}) and MDGP (0.994^{**}). Similarly, the MDGP trait showed the highest positive and significant correlation with SL (0.809^{**}) for the plant *Chenopodium album* (Fig. 5).

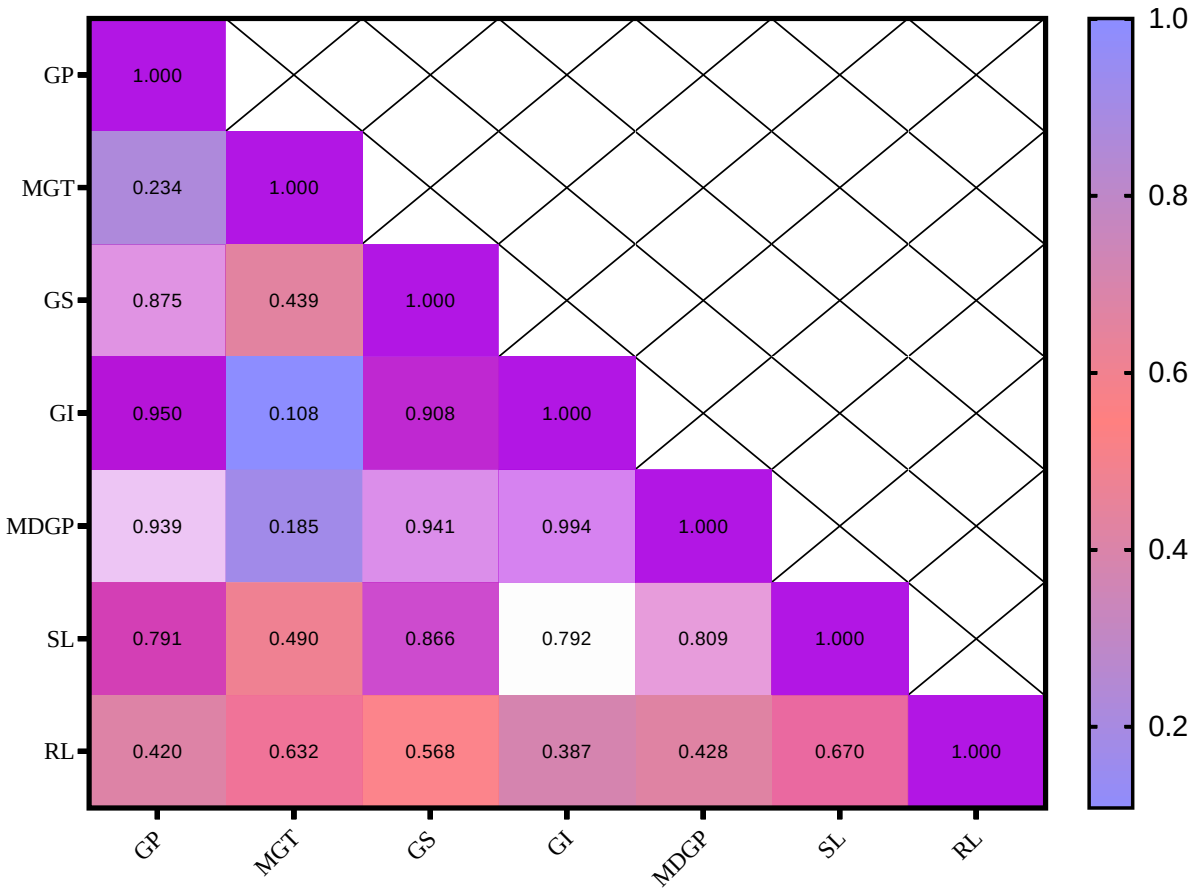

Fig. 5. Heatmap of the relationships between variables in the correlation coefficient for the traits studied in *Chenopodium album*.

This study aimed to determine whether the methanolic extracts of *Plumeria* and *Calotropis* could inhibit the germination and growth of other weeds. The results shown in Tables 4-10 revealed that the combined extract exhibited the most significant inhibitory effect on both seed germination and seedling growth of the plants examined. It is noted that extracts from these medicinal plants often contain various components, resulting in differing effects (Zhang *et al*., 2010, Haidarizadeh *et al*., 2024, Hosseini-Moghaddam *et al*., 2024). Numerous studies (Soltys *et al*., 2013, Hussain *et al*., 2011). indicate that allelopathic substances are secondary metabolites produced by plants, primarily through the shikimic acid and isoprene metabolic pathways. The most commonly recognized allelopathic substances include phenols, quinones, coumarins, flavonoids, terpenes, sugars, glycosides, alkaloids, and non-protein amino acids (Zhang *et al*., 2011, Li *et al*., 2021). Medicinal plants contain a wealth of secondary metabolites, such as essential oils, flavonoids, polysaccharides, tannins, terpenes, and trace elements (Cao *et al*., 2021).

Plumeria and *Calotropis* showed diverse inhibitory effects on selected weeds. However, the methanolic extract demonstrated varying inhibitory effects on different weed species. The combined treatment of 5% *Plumeria* + 10% *Calotropis* for *Chenopodium album* and the 10% *Plumeria* + 5% *Calotropis* treatment for *Onobrychis sativa* significantly inhibited the water extract in the tests, while its effect on *Amaranthus retroflexus* was very weak (Table 4). Similarly, the sensitivity order of each plant was (*Onobrychis sativa* > *Chenopodium album* > *Tribulus terrestris* > *Rumex acetosella* > *Amaranthus retroflexus*) (Tables 3 and 4). Allelochemicals inhibit the growth and development of plants (Staszek et al., 2021). Alkaloids, like phenolics, delay seedling growth by disrupting metabolic activities, leading to reduced growth and decreased dry matter accumulation (Arsana *et al*., 2022, Dias, 2024). Under severe conditions, phytotoxins limit seed germination (Adhikari *et al*., 2018). Cellular expansion and division are influenced by phytotoxins, which disrupt metabolic activities and lead to reduced seedling length. This study demonstrated that root growth is more sensitive to allelopathic stress than shoot growth, a finding that has also been supported by other research (Patanè *et al*., 2023). These results indicate that different weeds exhibit varying sensitivities to the allelopathic inhibition caused by the combined treatment of *Plumeria* and *Calotropis*. Currently, commercial herbicides are generally categorized into monocot and dicot herbicides. For example, Jinduer and Yanshu demonstrate better inhibitory effects on monocot weeds compared to dicot weeds, while butylate, 2,4-D, and Starane primarily target dicot weeds (Li *et al*., 2016). Our results indicate that the combined treatment of *Plumeria* and *Calotropis* effectively inhibits both monocot and dicot weeds, impacting not only seed germination but also seedling growth (Jmii *et al*., 2022). Therefore, the combined treatment of *Plumeria* and *Calotropis* can be used as a raw material for developing herbicides to effectively control weed growth.

The allelopathy of *Plumeria* and *Calotropis* offers a promising avenue for herbicide development. The increasing use of allelopathic inhibitors highlights their potential in creating plant-derived herbicides. Research indicates that the allelopathic potential of other plants may far exceed that of known allelopathic substances (Zhao *et al*., 2022, Shen *et al*., 2017), demonstrating effective herbicidal activity. Additionally, medicinal plants, which do not negatively impact agricultural crops, are worthy candidates for development as plant-based herbicides and present a viable alternative to certain chemical herbicides. Analyzing the chemical composition and exploring the allelopathic mechanisms of these studied plants provide a robust theoretical foundation for the ecological control of various weeds. However, further work is needed on

pesticide formulation techniques and broader field evaluations to fully realize this herbicide potential. Ultimately, our goal is to foster an ecological agricultural environment that promotes human health.

Conclusion

The results clearly demonstrate that the combined treatment had a significant allelopathic effect on seed germination and the overall growth of germinated seeds compared to the control treatment. Therefore, it is advisable to control weeds primarily by eradicating rhizomes or roots before sowing seeds. The phytotoxic properties of certain weeds can also be harnessed against other weed species, turning a harmful weed into a potential bio-herbicide. Given its rapid proliferation and toxic characteristics, this approach could be an effective strategy for biological control, and its abundance makes it economically viable. By adopting these practices, we can reduce our reliance on chemical herbicides and move toward more sustainable and safer agricultural methods.

Abbreviations

GP: Germination percentage
MGT: Mean germination time
GS: Germination speed
GI: Germination index
MDGP: Mean daily germination percentage
SL: Stem length
RL: Root length

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پتانسیل مهار عصاره برگ درختچه های زینتی *Plumeria rubra* و *Calotropis procera* بر جوانه زنی و رشد علف های هرز منتخب - راهی برای کاهش خطر علف کش های مصنوعی

چکیده

آللوپاتی پدیده ای جذاب است که در آن گیاهان ترکیبات شیمیایی آزاد می کنند که می تواند بر رشد و نمو گیاهان همسایه تأثیر بگذارد. این آللوشیمیایی‌ها می‌توانند جوانه‌زنی و رشد را تشویق یا مانع شوند و فرصتی هیجان‌انگیز برای ایجاد علف‌کش‌های بیولوژیکی دوست‌دار محیط‌زیست ارائه می‌دهند. شناسایی مواد گیاهی که به راحتی در دسترس، مقرون به صرفه و دارای خواص آللوپاتیک قوی هستند، می تواند مزایای زیست محیطی قابل توجهی به عنوان علف کش های بیولوژیکی داشته باشد. مطالعات متعدد قبلاً نشان داده اند که برخی از آللوشیمیایی های مشتق شده از گیاهان می توانند به طور موثر رشد علف های هرز را سرکوب کنند. در این مطالعه، اثرات آللوپاتیک عصاره دو درختچه زینتی گرمسیری Plumeria rubra و Calotropis procera را از طریق آزمایش‌های آزمایشگاهی بررسی کردیم. اثرات آللوپاتیک این عصاره‌ها بر جوانه‌زنی بذر و سایر صفات جوانه‌زنی به طور سیستماتیک مورد تجزیه و تحلیل قرار گرفت. یافته‌ها نشان داد که کاربرد ترکیبی ۵ درصد پلومریا + ۱۰ درصد استبرق برای Chenopodium album بسیار مؤثر بود، در حالی که تیمار ۱۰ درصد پلومریا + ۵ درصد استبرق برای *Onobrychis sativa* بیشترین تأثیر را داشت و منجر به مهار کامل جوانه‌زنی شد. در مقابل، عدم وجود عصاره باعث کاهش اثرات تنش و افزایش سرعت جوانه زنی شد. بیشترین درصد جوانه زنی برای *Onobrychis sativa* (55.00±5.010) ، *Rumex acetosella* (3.741±75.00)، *Amaranthus retroflexus* (5.849±100.00)، *Tribulus terrestris* (21.67±2.33±2.415) و *Chenopodium* (3.8±21.67) مشاهده شد. پیشنهاد شد که کاربرد ترکیبی ۵ Plumeria + 10% Calotropis % به طور قابل توجهی از جوانه زنی علف های هرز مانند Chenopodium album و Onobrychis sativa جلوگیری کرد. در نتیجه، این مطالعه تأیید کرد که استفاده ترکیبی از عصاره‌های متانولی به عنوان یک ماده خام بالقوه برای توسعه علف‌کش‌های پیش رویشی با هدف قرار دادن علف‌های هرز مختلف، نویدبخش است. این یافته مهم است زیرا شواهد علمی ارائه می دهد که می تواند از تحقیقات و توسعه آتی علف کش های سازگار با محیط زیست پشتیبانی کند.

کلیدواژه ها: استبرق، پلومریا، مهار، جوانه‌زنی، علف‌های هرز