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Exploration of the Allelopathic Effect of (*Euphorbia prostrata Aiton*) on the Germination and Growth of Alfalfa (*Medicago sativa*) and Two Weed Species

استكشاف التأثير التثبيطي لنبات اللُبينة (*Euphorbia prostrata*) على إنبات ونمو نبات البرسيم الحجازي (*Medicago sativa*) ونوعين من الأعشاب الضارة.

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Abstract:
The present study offers an applied contribution to the investigation of the allelopathic effects of the (*Euphorbia prostrata*) on seedling germination and growth of alfalfa (*Medicago sativa*) and Two Weed Species . The methanolic *Euphorbia prostrata* extract significantly affected the germination of seeds of *Medicago sativa, Setaria verticillata* and *Chenopodium Murale*. At a concentration of 10 g/L of the *Euphorbia prostrata* extract, complete inhibition of germination of *Medicago sativa* and *Chenopodium Murale* was recorded. While the percentage of seed germination inhibition was recorded as 88.15% for *Setaria verticillata* at a concentration of 10 g/L. However, the *Euphorbia prostrata* extract affected the percentage of root growth inhibition of plants and the results revealed slight differences. At a concentration of 10 g/L of the *Euphorbia prostrata* extract, the highest percentage of root growth inhibition was recorded (97.94%, 94.32% and 98.18%) for *Medicago sativa, Setaria verticillata* and *Chenopodium Murale*,

respectively . Allelopathic effect of *Euphorbia prostrata* extracts on shoot growth inhibition rate of *Medicago sativa, Setaria verticillata* and *Chenopodium Murale*. At 10 g/L concentration, the complete inhibition rate was observed for *Setaria verticillata* and *Chenopodium Murale*. While the highest shoot growth inhibition rate was 94.80% for *Medicago sativa*.

Key words: **Allelopathic**, **seedling germination and growth** ,***Euphorbia prostrata***, ***Medicago sativa***.

الملخص

الدراسة الحالية هي مُساهمة تطبيقية في الكشف عن التأثير الأليلوباثي لنبات اللبينة (*Euphorbia prostrata* Aiton) على إنبات ونمو شتلات محصول البرسيم (*Medicago sativa*) ونوعين من الأعشاب الضارة. أثر مستخلص الميتانولي لنبات *Euphorbia prostrata* بشكل كبير على إنبات بذور *Medicago sativa* و *Setaria verticillata* و *Chenopodium Murale*. يلاحظ عند تركيز 10 جرام/لتر من مستخلص *Euphorbia prostrata*، تم تسجيل تثبيط كامل لإنبات *Medicago sativa* و *Chenopodium Murale*. بينما تم تسجيل نسبة تثبيط إنبات البذور بنسبة 88.15% لنبات *Setaria verticillata* عند تركيز 10 جرام/لتر. ومع ذلك، أثر مستخلص *Euphorbia prostrata* على نسبة تثبيط نمو الجذور للنباتات وكشفت النتائج عن اختلافات طفيفة. عند تركيز 10 جرام/لتر من مستخلص *Euphorbia prostrata*، تم تسجيل أعلى نسبة لتثبيط نمو الجذور (97.94%, 94.32 %, 98.18%) لنباتات *Medicago sativa* و *Setaria verticillata* و*Chenopodium Murale*، على التوالي. التأثير الأليلوباثي لمستخلصات *Euphorbia prostrata* على معدل تثبيط نمو البراعم لنباتات *Medicago sativa* و *Setaria verticillata* و *Chenopodium Murale*. عند تركيز 10 جرام/لتر، لوحظت نسبة تثبيط كاملة لنمو *Setaria verticillata* و *Chenopodium Murale* بينما كانت أعلى نسبة تثبيط لنمو السويقات 94.80% لنبات البرسيم *Medicago sativa*.

الكلمات المفتاحية: التثبيط، إنبات البذور، النمو، نبات اللبينة، نبات البرسيم.

Introduction:

Alfalfa (*Medicago sativa*) is considered an important crop that most countries depend on in raising animals due to its richness in nutritional elements such as plant proteins, carbohydrates and vitamins [1, 2]. It also adapts well to the environmental conditions of most countries with a moderate climate. Some countries call it the "king of fodder" due to its high productivity and success in meeting the nutritional needs of animals in addition to its role in the fertility of agricultural soil [1, 2, 3].

Libya is one of the countries that depend on this crop in raising cows, sheep and camels, as most regions, especially the southern ones, cultivate it on a large scale and use it either fresh or dried [4].

This investigation focused on Allelopathic Effect of the Invasive (*Euphorbia prostrata*) on Seedling Germination and Growth of Alfalfa Crop (*Medicago sativa*) and Two Weed Species species (*Euphorbia prostrata* Aiton) of the genus Euphorbia. *E. prostrata* is one of the invasive weeds of the alfalfa crop in Samnu Oasis, southwest of Libya, which causes a decrease in production. Alfalfa (*Medicago sativa*).

This occurs through what is known as allelopathy, a phenomenon in which certain plants release specific chemicals into the environment, directly or indirectly affecting the germination or growth of neighboring [5] . defines allelopathy as any effect, whether inhibitory or stimulatory, arising from the release of specific chemical compounds into the environment by a particular plant or microorganism [6]. Smith and Martin contended that these molecules, referred to as secondary metabolites, allelochemicals, natural products, or phytoantagonists, significantly influence the composition of phytotic communities [7]. Hoagland asserted that numerous natural compounds can function as herbicides or serve as components in the formulation of novel herbicides [8].

Euphorbia prostrata is small annual herb belonging to Euphorbia genus , Family Euphorbiaceae. The species that are under the genus Euphorbia are among the most complicated and the largest groups of angiosperms on the planet. It has about 2000 species [8]. Some species that belong to the genus Euphorbia are known to be beneficial in the treatment of warts, migraines, and intestinal worms, among other diseases. Most of the species release large amounts of white poisonous latex after being cut[9,10,11].

The geographic range of this genus, which contains many succulents that resemble cacti, is the temperate climatic regions. The greatest concentration of succulent euphorbias exists in South and East Africa, Madagascar, temperate Asia, and the Americas [12]. Euphorbia represents one of the most substantial genera in the plant kingdom, comprising approximately 2008 species, as reported by Rajeswari The

genus Euphorbia has a range from annual plants to trees, demonstrating a practically global distribution, particularly in tropical, sub-tropical, and warm-temperate regions [13]. In Libya, 28 species of the genus Euphorbia were recorded [14]. *Euphorbia prostrata* found all over India. It is native to the west India and certain parts of south America and also widely naturalized in many other parts of the world [15]. In Libya it is found in cultivated lands and orchards. The active principles in *Euphorbia prostrata* are chiefly flavonoids, phenolic acids and tannins.

The aims of the present study were designed to investigate one of the invasive weeds affecting alfalfa crops and to evaluate the allelopathic potential of methanolic extracts of *Euphorbia prostrata* at various concentrations on the germination and growth of alfalfa (*Medicago sativa*) as well as two weed species in a laboratory setting.

Importance of the Study

This study is significant as it deepens our understanding of plant interactions, particularly concerning weeds and their effects on alfalfa production. Additionally, it addresses the gap in research related to the alfalfa crop in Libya, where we similar studies are lacking.

Material and methods:

Through field visits, from Jan. 2022 to Dec. 2023 we observed that *Euphorbia prostrata* interferes with *Medicago sativa*. It seems that allelopathy is an important factor in the interference caused by *E. prostrata* against *Medicago sativa* in the Samnu oasis, located in southwest Libya.

Euphorbia prostrata is a small annual herb, branched prostrate with many stems spreading from the roots, slender upto 20 cm long, leaves green but occasionally purplish red.

Plate (1): a- General view of *Euphorbia prostrata* Aiton in cultivated lands of alfalfa crop


Plate (1): b– General view of *Euphorbia prostrata* Aiton in waste ground

We performed field visits to multiple alfalfa crop fields in the Samnu oasis, located in southwest Libya, where we gathered *Euphorbia prostrata* Aiton plant specimens from diverse locations for identification and analysis. Following the collection of plant samples, they were transported to the Laboratory of the Biology Department at the Faculty of Education, Al-Jufra University. *E. prostrata* was meticulously cleaned, repeatedly washed with distilled water, dried in an oven at 50°C, ground into a fine powder, and stored in a tightly sealed bottle for subsequent analyses. The experiment was conducted twice, and the average data were calculated.

Allelopathic potentiality of *Euphorbia prostrata* Aiton

We collected *Euphorbia prostrata* Aiton to study its allelopathic effect on the germination and growth of the alfalfa crop (*Medicago sativa*) and two weed species. Field observations and literature reviews guided the selection of the chosen species. Seeds of *Setaria verticillata* and *Chenopodium murale* were collected from different locations in Samno farms in southwestern Libya, while Medicago sativa seeds were obtained from a store for selling seeds. We sterilized them with 0.3% calcium hypochlorite solution, rinsed with distilled water, and dried them in the shade on filter paper in the laboratory at room temperature for 7 days [17].

Preparation of the botanical specimen

Plant cuttings during the vegetative growth stage were cleaned by rinsing in distilled water and air-dried at room temperature in a shaded place for several days until complete desiccation was achieved. When completely desiccated, the samples were pulverized and sieved through a 1 mm sieve and kept in the fridge.

Preparation of methanol extract

Ten grams of dried sample was placed in a 100 ml conical flask with 30 ml methanol, capped with cotton, and placed in a water bath at 60 oC for 30 minutes, allowing for continuous stirring. The extracts were then cooled down to room

temperature, filtered, and subjected to concentration with the aid of a rotary evaporator. For other bioassay evaluations, stock solutions of 10% (w/v) were prepared in addition to other concentrations of 2.5%, 5%, 7.5%, and 10% (v/v) electrolytes of Euphorbia extractsAll bioassay solutions, applied at osmotic concentration below 0.1 MPa, were assumed not to influence the germination percentage [18]. The solutions were filtered into a glass container covered with double layers of muslin and possessed a starting plan as a 1 filter paper. The pH was adjusted with 1M HCl at 7 prior to storing at low temperatures (below 5 degrees) until the analysis was conducted [6].

Bioassays for Germination

We placed two layers of Whatman No. 1 filter paper in 90 mm diameter glass laboratory Petri dishes to conduct the experiments. Each dish held twenty seeds and 10 ml of *Euphorbia prostrata* extract, varying in concentrations from 2.5% to 10% (v/v), and kept all at room temperature.. Observations were made daily on all those planted from 1 day after the experiment, and germination was determined, when a seed shows cotyledons extending to a length of 0.5 cm. The randomization process was a randomized complete block (RCB), with three replications. The process was repeated twice. The germination percentage is determined by dividing the number of germinated seeds for each day to the number of days and summing the total. Inhibition % was determined using the formula: Inhibition % = [(CG-TG) / CG] x 100 Where CG is the germination percentage of the control treatment, with TG signifying the germination percentage of the extract treatment.

Growth bioassays

The seeds of *Medicago sativa*, *Setaria verticillata* and *Chenopodium murale*.were germinated on filter paper in darkness at ambient temperature for 48 hours. Fifteen germinated seeds were placed in Petri dishes containing 25 g of quartz sand and 10 ml of shoot powder extract at concentrations of 2.5%, 5%,7.5% and 10% (v/v).

A control was included in the experiment without any powder treatment. The experiment was constructed as a Randomized Complete Block (RCB) with three replicates, and it was conducted twice. The shoot and root lengths of seedlings were measured 14 days after treatment (DAT), and growth inhibition in shoot and root lengths was estimated using the following equation:

Growth inhibition = [(LC–L.T) / LC] ×100

Where, growth inhibition is expressed as a percentage; L.T denotes the shoot or root length of powder-treated weeds; L.C represents the shoot or root length of untreated control weeds.

Study Methodology

In this study, we employed the experimental method to design and implement the research according to its requirements.The laboratory investigation utilized a completely randomized design (CRD), and statistical analysis was performed to evaluate variance using ANOVA. Averages were compared at a significance level of P ±0.05.

Results and discussion:

Germination bioassays

The methanolic extracts of *Euphorbia prostrata* exerted an allelopathic effect on the germination inhibition percentage of *Medicago sativa, Setaria verticillata* and *Chenopodium murale*. four days after treatment (DAT) are shown in Table (1) and Figure (1). At the concentrations 2.5, 5, 7.5 and 10 g/l, the inhibition percentages were 15.83%, 55.75%, 98.23% and 100%, respectively for *Medicago sativa* 8.46%, 34.22%, 76.11% and 88.15 %, respectively for *Setaria verticillata* And the inhibition percentages were 18.25%, 73.81%, 100% and 100% respectively for *Chenopodium murale*, obtained data from (table 1, figure 1), revealed that there were not any significant variations (LSD =21.45) for averages. When the concentration of *Euphorbia prostrata* extracts increased, then the degree of inhibition increased. At the 10 g/l concentration of *E. prostrata*, there was a complete inhibition of the germination of *Medicago sativa*. While the highest inhibition percentage on the germination of *Setaria verticillata* is 88.15% At the 10 g/l concentration of *E. prostrata* and at the (7.5, 10 g/l) concentration of *E. prostrata*, there was a complete inhibition of the germination of *Chenopodium murale*.

Table (1). Effect of methanolic extracts of Euphorbia prostrata on the germination inhibition percentage (mean value ± standard error) of *Medicago sativa*, *Setaria verticillata*, and *Chenopodium murale* after 4 DAT.

Plant species	Extract of *Euphorbia prostrata*			
	Concentration (g/l)			
	2.5	**5**	**7.5**	**10**
Medicago sativa	15.83±0.73	55.75±0.46	98.23±0.67	100.00±0.0
Setaria verticillata	8.46±1.15	34.22±0.78	76.11±0.33	88.15±0.33
Chenopodium murale	18.25±0.47	73.81±0.77	100.00±0.0	100.00±0.0

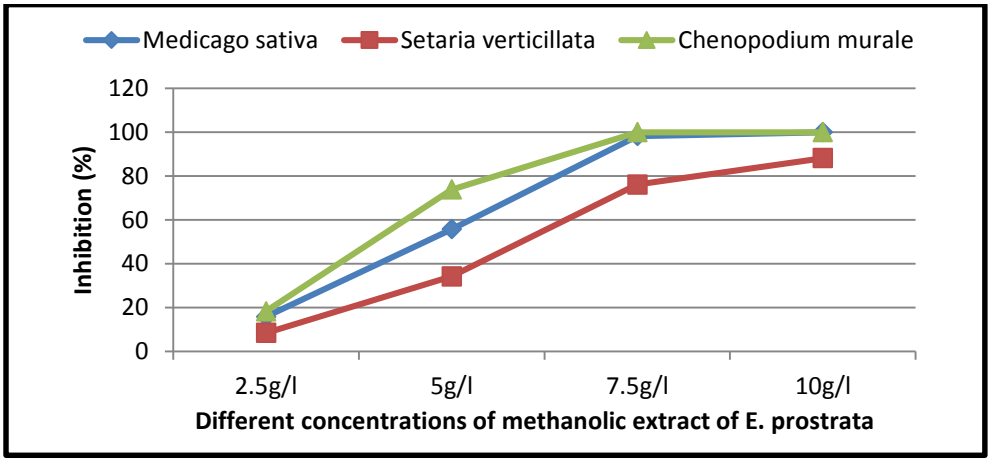

Figure (1). Effect of methanolic extracts of *Euphorbia prostrata* on the germination inhibition percentage of the *Medicago sativa* , *Setaria verticillata* and *Chenopodium murale* after 4DAT.

Growth bioassays

Root growth inhibition

The allelopathic effect of *Euphorbia prostrata* extracts on the root growth inhibition percentage of *Medicago sativa* revealed that there were slight significant variations (LSD= 5.33) for averages. The concentration of *Euphorbia prostrata* extracts increased as the degree of inhibition increased. At the 10 g/l concentration of *E. prostrata*, , *Setaria verticillata* and *Chenopodium murale* after fourteen days treatment (DAT) are shown in Table (2) and Figure (2). At the concentrations2.5, 5, 7.5 and 10 g/l, the inhibition percentages were 91.26%, 95.38%, 96.11% and 97.94%, respectively for *Medicago sativa*, 72.13%, 86.94%, 90.01% and 94.32%, respectively for *Setaria verticillata*, 94.81%, 96.89%, 97.05% and 98.18%, respectively for *Chenopodium murale*.

Table (2). Effect of methanolic extracts of *Euphorbia prostrata* on the root growth inhibition percentage (mean value ± standard error) of *Medicago sativa*, *Setaria verticillata* and *Chenopodium murale* after 14DAT.

Plant species	Extract of *Euphorbia prostrata*			
	Concentration (g/l)			
	2.5	5	7.5	10
Medicago sativa	91.26±0.33	95.38±0.47	96.11±0.03	97.94±0.03
Setaria verticillata	72.13±0.03	86.94±0.23	90.01±0.33	94.32±0.33
Chenopodium murale	94.81±0.25	96.89±0.07	97.05±0.33	98.18±0.09

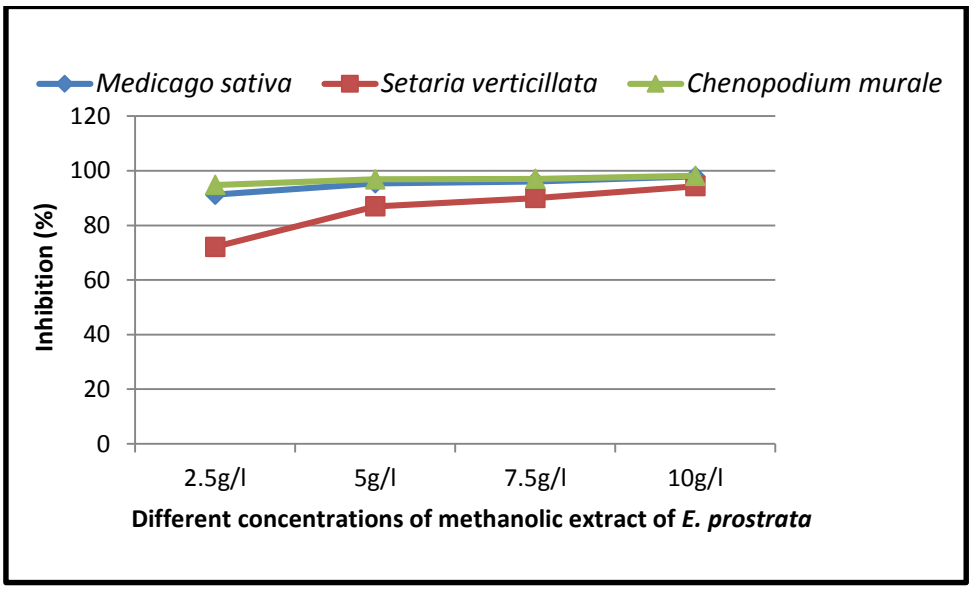

Figure (2). Effect of methanolic extracts of *Euphorbia prostrata* on the root growth inhibition percentage of *Medicago sativa*, *Setaria verticillata* and *Chenopodium murale* after 14DAT.

Shoot growth inhibition

The allelopathic effect of *Euphorbia prostrata* extracts on the shoot growth inhibition percentage of *Medicago sativa*, *Setaria verticillata* and *Chenopodium murale* after fourteen days treatment (DAT) are showed in Table (3) and Figure (3). At the concentrations 2.5, 5, 7.5 and 10 g/l, the inhibition percentages were 42.19, 71.01, 80.09 and 94.80%, respectively for *Medicago sativa,* 34.13, 59.05, 78.23 and 100 %, respectively for *Setaria verticillata,* 59.38, 75.18, 100 and 100%,

respectively for *Chenopodium murale*.Also result revealed that there were slight significant variations (LSD= 8.07) for averages.

Table (3). Effect of methanolic extracts of *Euphorbia prostrata* on the Shoot growth inhibition percentage (mean value ± standard error) of *Medicago sativa*, *Setaria verticillata* and *Chenopodium murale* after 14 DAT.

Plant species	Extract of *Euphorbia prostrata*			
	Concentration (g/l)			
	2.5	**5**	**7.5**	**10**
Medicago sativa	42.19±0.23	71.01±0.57	80.09±0.10	94.89±0.17
Setaria verticillata	34.13±0.33	59.05±0.17	78.23±0.15	100.00±0.00
Chenopodium murale	59.38±0.17	75.18±0.28	100.00±0.00	100.00±0.00

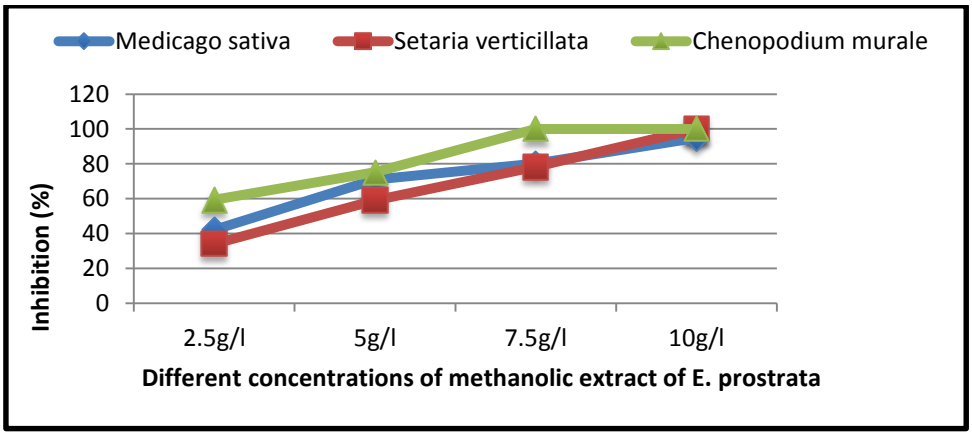

Figure (3). Effect of methanolic extracts of Euphorbia prostrata on the shoot growth inhibition percentage of *Medicago sativa*, *Setaria verticillata* and *Chenopodium murale* after 14 DAT.

Discussion:

The current research explores the impact of the one invasive plant species *Euphorbia prostrata* on the germination and growth of alfalfa (*Medicago sativa*) seedlings as well as two weed species. The study analyzes the synergistic effects on the ratios of germination inhibition, shoot growth inhibition, and root growth inhibition. This investigation significantly enhances the understanding of the synergistic interactions by examining the direct effects of the invasive *Euphorbia prostrata* on *Medicago sativa, Setaria verticillata,* and *Chenopodium murale.*

Numerous specialists have highlighted the challenges posed by Euphorbia species, which significantly hinder seedling germination and initial crop establishment, thereby limiting agricultural output.

On other hand, several plant species of the family, Euphorbiaceae have been demonstrated to have herbicidal and insecticidal properties. But that require further confirmation of their commercial potential as eco-friendly plant protection agents. This research provides important insight into the important role that invasive species have in shaping agricultural ecosystems and natural communities. This means that Euphorbia might also a good source in herbicide production. Allelopathic compounds, being secondary metabolites acted from an emission out of the plant by volatilization, leaching, root excretion and in the decomposition of plant material in organic spectrum. Metabolite classes such as phenolics, flavonoids, alkaloids, terpenoids, and cyanogenic glycosides have captured scientific interest for their structures and biological roles [18]. Aslam et al., have reported the composition of Euphorbia which comprises carbohydrates, saponins, tannins, phenols, quinones, proteins, terpenes, glycosides and carbohydrates[19]. While, Nirmala and Rakesh , identified that, glycosides, flavonoids, polyphenols, phytosterols, and tannins in *Euphorbia prostrata* contribute to their significant allelopathic behaviour on plants[20].

Euphorbia extracts inhibited with a concentration-dependent pattern. How competitive a particular weed is in given conditions is determined by the accompanying crops and by interactions with other weed species [21]. The extract of *Euphorbia prostrata* was shown to significantly affect the germination of seeds of *Medicago sativa, Setaria verticillata* and *Chenopodium Murale* in this study. The *E. prostrata* extract at a concentration of 10 g/L completely inhibited the germination of the *M. sativa* and *C. Murale* extracts. Also, *S. verticillata* had a germination inhibitory percentage of 88.15% at same concentration.

Recapsulating the extract adjusted percentage of root growth inhibition varied slightly in results. Root growth inhibition rates at 10 g/L were as high as 97.94%, 94.32%, and 98.18% for *M. sativa, S. verticillata,* and *C. Murale*, respectively. *Euphorbia prostrata* showed strong allelopathic potential reflecting on the shoot

growth inhibition percentage of *M. sativa, S. verticillata,* and *C. Murale*. In *S. verticillata and C. Murale*, total inhibition was recorded at the concentration of 10 g/L, and *M. sativa* displayed a maximum shoot growth inhibition of 94.80%. The phytotoxic properties of Euphorbia extracts have been the subject of numerous studies, which were in accordance with the results of this study. The differential allelopathic responses of different plants are determined by the existing distribution and concentration of allelopathic constituents over the different part of herb [22,23]. Drunken that the effect of the *E.hirta* on the maize was concentration-dependent, low concentrations promote the growth of maize seedlings [22]. The roots, stems, leaves and fruits extracts of *E. helioscopia* also inhibited the chickpea, lentil and wheat seeds germination with the most inhibition observed with leaves extract[24,25]. Tanveer et al noted that species of Euphorbia can reduce plants in pastures by 10% to 100%. [26].

Conclusion:

Finally, The results study showed that, *Euphorbia prostrata* extracts hindered alfalfa (*Medicago sativa*) germination and growth, posing challenges for seedling establishment and alfalfa production. This underscores the need for science-based weed management strategies. Conversely, these extracts may serve as eco-friendly alternatives to chemical herbicides, affected the germination and growth of *Setaria verticillata* and *Chenopodium murale*. However, further research is necessary to assess their effectiveness and cost-efficiency for sustainable agriculture.

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