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Assessment of Allelochemicals Potential of *Peganum harmala* L. for Biological Control of *Cynodon dactylon* L.

Amal Ehtaiwesh[1] and Latifa Yarboa2

[1] *Plant science Department, Science Faculty, Zawia University, Libya*
[2] *Veterinary Medicine and Agricultural Sciences Faculty, Zawia University, Libya*

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A B S T R A C T

This study was conducted to evaluates the allelopathic potential of *Peganum harmala L.* (Zygophyllaceae) on the growth of Cynodon dactylon L. (Poaceae). Seeds, stems, and a mixture of (seeds and stems) aqueous extracts of *P. harmala* plants were applied as a foliar application to three weeks old seedlings of *Cynodon dactylon* weeds. The result indicated that the application of aqueous extracts of different plant parts of *P. harmala* caused a significant reduction in growth traits of *Cynodon dactylon* including plant height, number of branches, number of leaves, and plant weight. Moreover, the result revealed that the Inhibitory effect of *P. harmala* aqueous extract on Cynodon dactylon L was more pronounced in plant height and number of leave plant-1 traits. In addition, the results illustrated that among the different *P. harmala* plant parts used in this study, seeds extract was the most toxic and caused the greatest negative effect on growth traits of Cynodon dactylon. According to the results, the degree of toxicity of different P. harmala plant parts can be arranged in the following order: seeds > mixture > stems. The results of this study concluded that the use of *Peganum harmala* L plant has the potential to be developed further as a bio-herbicide system to control weed such as *Cynodon dactylon* L.

Introduction

Weeds are one of the major causes of economic losses in crop production worldwide (Khan et al., 2020). Cynodon dactylon is among the 20 most globally widespread weeds (Mubeen et al., 2011). In many regions worldwide, weeds inflict 20-30% losses in different crops on average (Hussain et al., 2007). Cynodon dactylon is one of the serious weeds in Libya as well as many regions around the globe. The grass is usually controlled through a variety of management strategies, such as traditional tillage, hand weeding, mechanical cultivation, cultural practices, or chemical herbicides (Shemdoe et al., 2008; Ninsuwan et al., 2011). Worldwide, enormous quantities of chemicals herbicides are applied to manage these weeds. However; artificial chemical herbicides might be toxic and may harm the environment (Khanh et al., 2004; Macías et al., 2006). Also, chemical herbicides may cause human health problems, cause groundwater contamination, and may cause toxicity in foods (Hong et al., 2004). In addition, the over-applying of herbicides has led to the development of weed biotypes with herbicide resistance (Batish *et al.*, 2007). Therefore, to reduce dependence on synthetic chemicals herbicides, the use of plants that possess strong allelopathic properties has shown promising results for weed control (Hong *et al.*, 2004). Inhibitors substances released from allelopathic plants can control weeds in a sustainable manner and reduce labor costs.

**** Corresponding author:***
E-mail: a.ehtaiweshl@zu.edu.ly
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Allelopathy as defined by the International Allelopathy Society "any procedure involving secondary metabolites produced by plants, algae, bacteria, and fungi that influence the growth and development of agricultural and biological systems" (Hussain *et al*., 2007). The allelochemicals can be generally classified as plant phenolics, and terpenoids which show great chemical variety and are involved in a number of metabolic and ecological processes (Xuan *et al.*, 2005). Allelochemicals are toxic phenols, terpens and alkaloids and may inhibit or retard seed germination, inhibit shoot and root growth, reduce nutrient uptake, reduced dry weight accumulation and lowered reproductive capacity of different plants (Abu-Romman *et al*., 2010) Many allelopathic and medicinal plants have been revealed and confirmed to regulate agricultural weeds and therefore might be used as an alternative for weed control (Dayan and Duke, 2006). All the plant parts could display allelopathic effects and may significantly affect nearby plants, however the allelopathic effects of various parts of the same plant vary for their effects on the growth of plants (Ashrafi *et al*., 2008; Khan *et al*., 2020; Poonpaiboonpipat *et al*., 2021).

Harmal *(Peganum harmala* L.) grows in arid and semi-arid regions include central Asia, Middle East, and North Africa (Mahmoudian *et al.*, 2002; Zhao *et al.*, 2011). Harmal plants have been used as a medication to treat many illnesses such as coughs, diabetes, asthma and to relieve dolorous process (Kartal *et al.,* 2003; Farouk *et al.,* 2008). Hermal contains different secondary metabolites which can affect plant growth (Sodaeizadeh *et al*., 2009). Many studies indicated that Harmal plant possess allelopathic properties and many chemicals such as alkaloids, steroids, flavonoids, anthraquinones, polysaccharides, and amino acids have been isolated from the plant (Chatterjee and Ganguly, 1968; Shanna *et al.,* 1982; Sharaf *et al.*, 1997; Movafeghi *et al.*, 2009). Other studies have reported that Harmal plants showed inhibitory effect on seed germination and plant growth of nearby plants such as ryegrass (Shao *et al.*, 2013), radish (Khan *et al.*, 2011), barley and wheat (Imran, 2014). However, to our knowledge, no study has been done to prove the phytotoxic effect of any medicinal plant against *Cynodon dactylon* L weeds. Therefore, the aim of this research was to assesses the herbicidal potential of *P. harmala* on growth of *Cynodon dactylon*.

2 Materials and Methods

This study was conducted in the Summer of 2020, in semi controlled environment facilities in Surman farm and at the Plant Science Department, University of Zawia, Libya.

2.1 Plant materials

P. harmala L seeds, stems, and the mixture of seeds and stems were obtained from the local market and *Cynodon dactylon* L were obtained from a farm in Zawia City. This study was conducted to evaluate the inhibitory potential of *P. harmala* seeds, stems, and a mixture of seeds and stems extract on germination and growth of *Cynodon dactylon* L weeds.

2.2 Preparation of Aqueous Extract

Aqueous extract of *Peganum harmala* seeds, stems and mixture of seeds and stems were prepared following the modified method (Sodaeizadeh et al., 2009). Fifteen grams of each plant part was separately extracted using 150 ml distilled water. Each plant part was mixed in a household blender for 20 minutes. Then extracts were filtered through Whatman No. 1 filter paper and used as spray to treat and *Cynodon dactylon* L plants.

2.3 Experimental and treatment conditions

The 10 cm in length seedlings of *Cynodon dactylon* L were sown in pots. 1/2 L Plastic pots were filled with peat moss and 2 plants of *Cynodon dactylon* were sown in each pot and irrigated with fresh water as needed. Three weeks after sowing plants were treated with *P. harmala* aqueous extracts. At the time pots were divided into four groups with four replications, each group represents one treatment which include control (no application of *P. harmala)*, seed, stems and a mixture of seeds and stems of *Peganum harmala* extract. Each set of pots were sprayed with corresponding *P. harmala* extract or water for the control set, and the spraying was repeated every week for a total of three times. Following the treatments, all pots were kept under semi- controlled conditions and irrigated manually to saturation as needed for two weeks. Plants were harvested about 60 days after sowing.

2.4 Data collection

At the end of the experiment, one plant from each pot was collected and data on plant height (cm), number of branches plant^{-1}, number of leave plant^{-1}, plant weight (g) was recorded. Weed control efficiency (WCE) was calculated based on the dry weight of weeds following the equation of (Hasanuzzaman et al., 2009)

$$\mathrm{WCE}(\%) = \frac{\mathrm{WDC}-\mathrm{WDT}}{\mathrm{WDC}}\ \mathrm{x}100.$$

Where, WDC = Weed seedling dry weight in control pot, WDT = Weed seedling dry weight in the treated pot.

Statistical analysis

The experimental design was a randomized complete design (RCD) with four replications. Analysis of variance performed using generalized linear model (GLM) procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Separation of means was carried out using the least significant differences (LSD; $P < 0.05$). The means were compared using Duncan's multiple range test.

3 Results

The Probability values for weed growth traits obtained in this study are presented in Table 1. The aqueous extract of seeds, stem, and a mixture of seeds and stems of *Peganum harmala* inhibited growth traits of *Cynodon dactylon*. Table 1 showed a high significant effect (p<0.001) of plant extracts on weed control efficiency for *C. dactylon*. In addition, the results indicated that *P. harmala* plant extracts significantly (p<0.01) effected plant height and number of leave plant^{-1} traits (Table 1). Similarly, the number of branches plant^{-1} and plant weight traits were significantly (p<0.05) effected by *P. harmala* plant extracts (Table 1).

Table 1. Probability values of the effects of *Peganum harmala* L. extracts on growth traits of *Cynodon dactylon* L weeds.

Traits	Probability values
Plant height (cm)	0.0042
Number of branches plant^{-1}	0.0295
Number of leaves plant^{-1}	0.0063
plant weight (g)	0.0134
Weed control efficiency (%)	0.0009

In addition, the result indicated that there were significant differences in the inhibitory effect between plant parts of *Peganum harmala* L. extract used in this study (Table2).

Table 2. The effects of *Peganum harmala* L. extracts on growth traits of *Cynodon dactylon* L weeds.

Traits	Different plant part extract			
	control	Stems	Mixture	Seeds
Plant height (cm)	33^{a}	25.75^{b}	24^{b}	21^{b}
Number of branches plant^{-1}	5.25^{a}	4.75^{a}	3.5^{ab}	2.5^{b}
Number of leaves plant^{-1}	45.75^{a}	39.25^{ab}	30.75^{bc}	27.25^{c}
plant fresh weight (g)	6^{a}	4.75^{ab}	2.75^{bc}	2^{c}
Weed control efficiency (%)	-	24^{b}	52^{a}	68.6^{a}

[*] Individual value is the mean of 4 plants under different plant part extract. Values followed by different letters are significantly different according to Duncan's multiple range test (*P < 0.05*).

The results showed that aqueous extract of stems, mixture and seeds of *P. harmala* decreased plant height % by 22%, 27%, and 36% respectively over control (Fig 1).

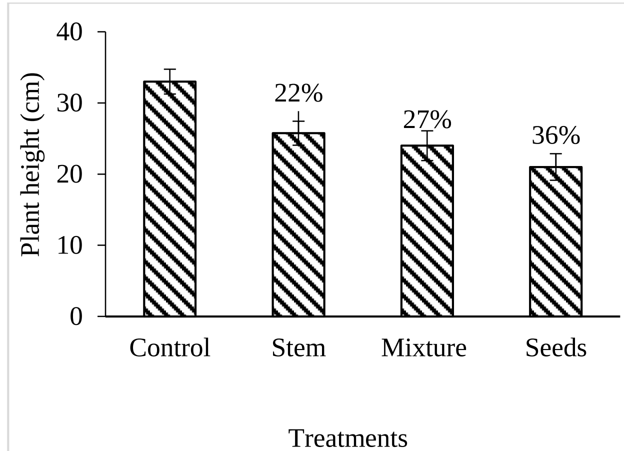

Figure: (1) The effects of *Peganum harmala* L. extracts on plant height trait of *Cynodon dactylon* L. weeds. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicate the percent reduction from control.

Also, the results showed that aqueous extract of stems, mixture and seeds of *P. harmala* decreased number of branches plant^{-1} of *Cynodon dactylon* by 10%, 33%, and 52% respectively over control (Fig 2).

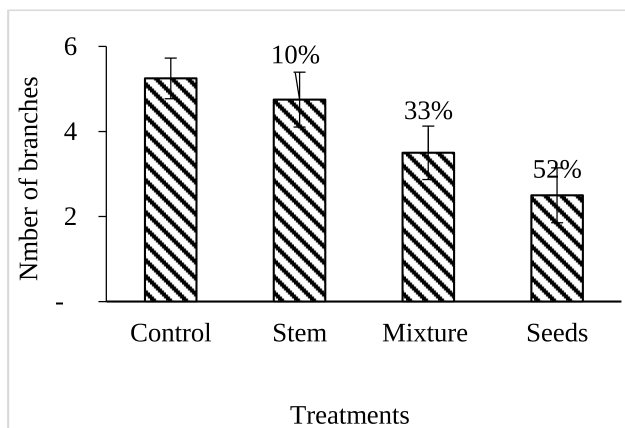

Figure: (2) The effects of *Peganum harmala* L. extracts on the number of branches traits of *Cynodon dactylon* L. weeds. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicate the percent reduction from control.

The same trend was found with the number of leaves plant^{-1}. Figure 3 showed that number of leave plant^{-1} decreased by 14%, 33%, and 40% due to the application of aqueous extract of stems, mixture and seeds of *P. harmala* respectively.

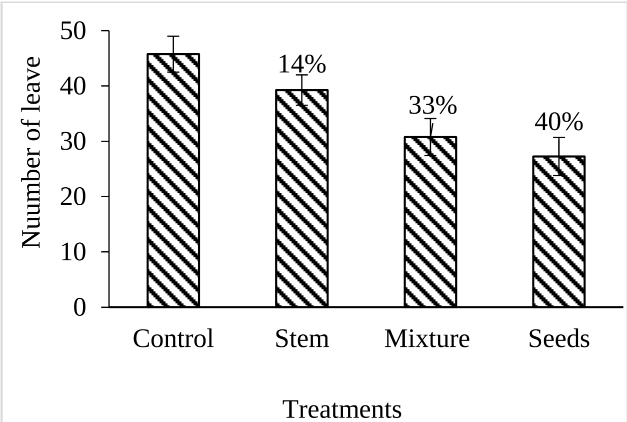

Figure: (3) The effects of *Peganum harmala* L. extracts on the number of leave traits of *Cynodon dactylon* L. weeds. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicate the percent reduction from control.

Moreover, the aqueous extract of *P. harmala* inhibited another growth trait of *C. dactylon.* Figure 4 showed that there was a significant decrease in plant weight of *C. dactylon* due to the application of *P. harmala* aqueous extract. The result indicated that aqueous extract of stems, mixture, and seeds of *P. harmala* decreased plant weight by 21%, 54% and 67% respectively Fig 4.

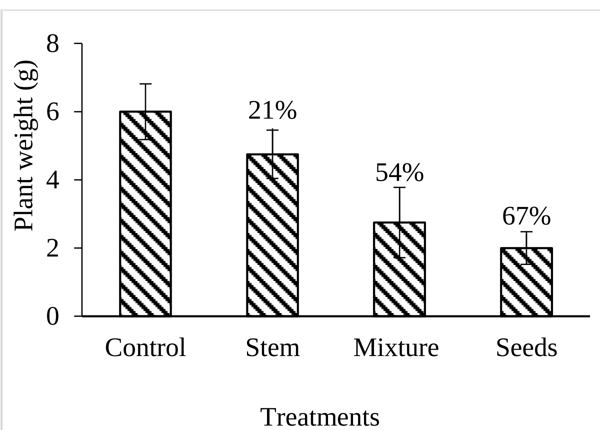

Figure: (4) The effects of *Peganum harmala* L. extracts on plant weight traits of *Cynodon dactylon* L. weeds. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicate the percent reduction from control.

Additionally, the results revealed that seeds extract of *P. harmala* resulted in higher weed control efficiency for Cynodon dactylon with control efficiency of 69%, which maybe as a result of active inhibition of weed growth. Whereas, a weed control efficiency of aqueous extract of the mixture and the stems for Cynodon dactylon reported with 24% and 52% respectively Fig5.

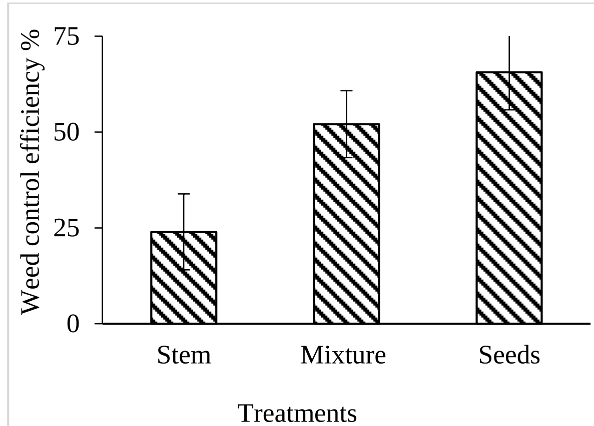

Figure:5) The effects of aqueous extract of *Peganum harmala* L extracts on weed control efficiency for *Cynodon dactylon* L. weeds. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4).

4 Discussion

In this study the evaluation of the herbicidal potential of Peganum harmala L. for biological control of Cynodon dactylon L. weeds were investigated. This investigation revealed that aqueous extracts of seeds, stem, and the mixture of seeds and stems of P. harmala reduced the growth traits of Cynodon dactylon L. (Table 2). All plant parts included in this study showed inhibitory effects on weed growth traits, which agree with previous studies that addressed the inhibitory effect of some plant species on neighboring plants (Turk and Tawaha, 2003; Xuan et al., 2004; Sodaeizadeh et al., 2009). These inhibitory effects may be caused by the phytotoxic potential of P. harmala. This finding agrees with other studies (Singh et al., 2003; Sodaeizadeh et al., 2009). Even though all plant parts (seeds, stems and mixture of seeds and stems) of P. harmala used in this study displayed inhibitory effects on weed growth traits, however, the study found that seeds of P. harmala exhibited the strongest inhibitory activities as compared with other plants parts, this finding was corroborated with the findings of (Shao et al., 2013; Poonpaiboonpipat et al., 2021). Many researches have been done on medicinal plants and came out with a number of plants that have chemicals appropriate for suppressing the growth and yield of surrounding plants like the aqueous methanol extract of L. aspera significantly inhibited the growth of seedlings of some plant species such as cress (Lepidum sativum L.), alfalfa (Medicago sativa L.), lettuce (Lactuca sativa L.), jungle rice (Echinochloa colonum L. Link) and timothy (Phleum pratense L.) (Islam et al., 2012). The result revealed that aqueous extracts of different parts of P. harmala plant (seeds, stems and, a mixture of seeds and stems) inhibited the growth traits of Cynodon dactylon weeds. This outcome is supported by other study which indicted that aqueous extract of *Ageratum conyzoides* inhibited radish germination (Xuan *et al.*, 2004). Also, the analysis of variance revealed significant differences among the aqueous extract of different parts of *P. harmala* plant on plant height (cm) of *Cynodon dactylon*. This result is in agreement with another study that indicated that *P. harmala* reduced plant growth of wheat and barley plants (Imran, 2014). Also, the result herein indicated the plant weight of *Cynodon dactylon* was effected by the application of aqueous extract of *P. harmala*. Other studies investigated the effect of an aqueous extract of different plants on growth and biomass accumulation of other plants found the same results for diverse crops such as: allelopathic effect of black mustard on wild Oat (Turk and Tawaha, 2003), aqueous extract of *A. conyzoides* reduced radish weight (Xuan *et al.*, 2004). Also the weight of *Cynodon dactylon* was significantly affected under aqueous extract application of *Senna angustifola* plants (Hussain *et al*., 2007). Likewise; the toxic effect of *Eucalyptus* spp on germination and growth traits of *Dactyloctenium aegyptium* (Ehtaiwesh, 2021) The present study concluded that *P. harmala* has a phytotoxic effect and inhibited the growth of *Cynodon dactylon*. Similar results were observed on the shoot and root length of wheat and mustard treated with different concentrations of aqueous plant extracts of *P. harmala* (Aslam *et al*., 2016). By these findings it is evident that *P. harmala* plants can produce phytochemical compounds with plant growth inhibitory activities. It appears that the allelopathic activity which usually causes delaying or maybe preventing growth and/or reduce plant growth traits is resulted from the original effects of these materials on the metabolic process of nearby plants.

5 Conclusion

Generally, the results suggest that *P. harmala* could be used as a natural herbicide for controlling *Cynodon dactylon* plants which may reduce the dependence on chemical herbicides. According to these results, the phytotoxic potential of *P. harmala* varied with different plant parts used to prepare the extracts. Among different plant parts used, seeds activated a greater growth inhibitor for *Cynodon dactylon* plants followed by the mixture of seeds and stems of *P. harmala*.

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Conflict of interest: The authors declare that there are no conflicts of interest.

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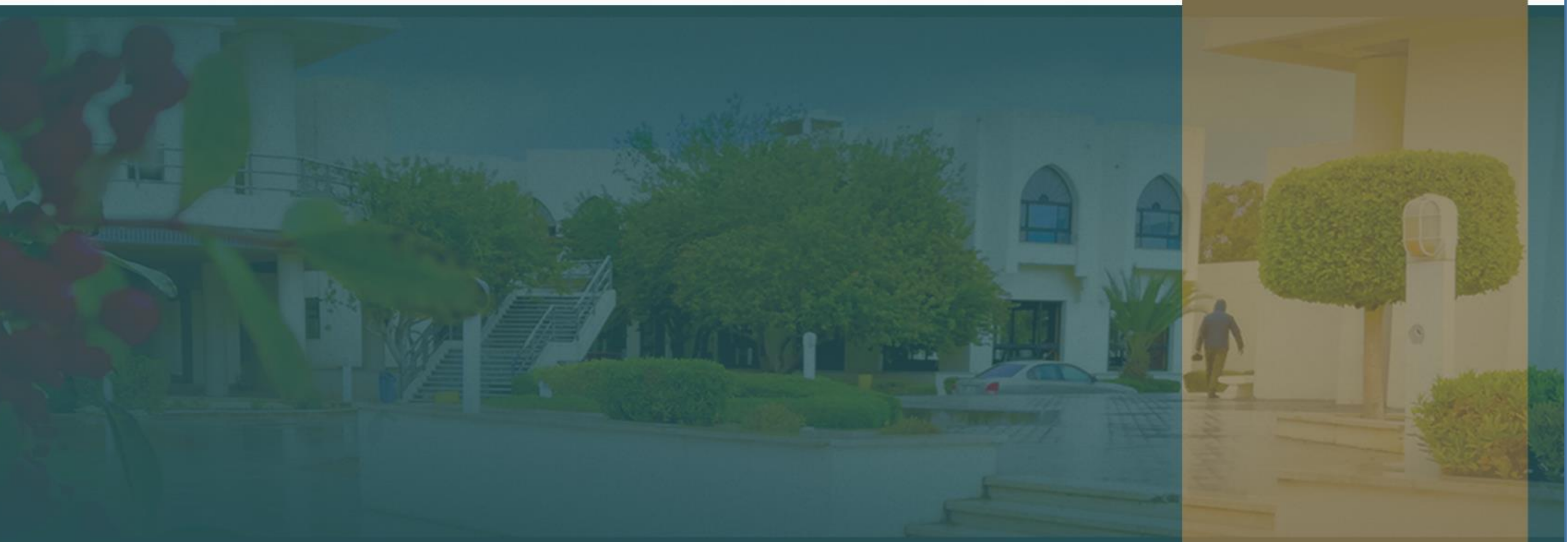

media92production@gmail.com

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