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AUTOTOXIC EFFECT OF *Anagyris foetida* L. ALKALOIDS CONTENT

Samar Emad Ezzat*

Al-Nissour University College, Al-Nisour University, Baghdad, Iraq

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KEYWORDS

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Anagyris foetida

Autotoxicity

Aporphine

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ABSTRACT

The present study aimed to evaluate the autotoxic effect of *Anagyris foetida* root, flower, and leaves alkaloids on the seed germination and growth of the same species. The pot experiment was conducted for three concentrations i.e. 4, 8, and 12% of aqueous extracts of root, flower, and leaves. Further, the seed and leaves were evaluated for their alkaloids content i.e. cytosine, aporphine, and anagyrine by high-performance liquid chromatography. The results of the study suggested that seed treated by 4% root extract showed a decrease in aporphine, cytosine, and anagyrine. While the seed treated with the flower and leaves extract (12%) showed a lower concentration of all three alkaloids as compared to untreated control. While a mixture of all three parts extracts at 4% concentration showed a minimum concentration of aporphine, cytosine, and anagyrine alkaloids in the treated seeds. *Anagyris foetida* roots effectively secrete allelopathic compounds which inhibit *A. foetida* plant germination and growth. The allelopathy effect of plants can be studied in the integrated management of poisonous plants. Results of the study suggested that the farmers should not repeat the *A. foetida* plantation continue two successive years.

* Corresponding author

E-mail: samar.e.anesth@nuc.edu.iq

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1 Introduction

An autotoxicity, a self-toxicity, is a biological phenomenon in which one species inhibit the reproduction/germination or growth of other plants that belong to the same family through the production or release of the specific chemical into the nearby environment (Ghimire et al., 2019; Zhang et al., 2021). The autotoxic compounds are released by the producer plant and it showed the effects on the receptor plant, i.e. effector, which is of similar types (Yu, 2001; Chon et al., 2004). Many crops like watermelon, cucumbers, pumpkins, etc. are involved in the autotoxic compound secretion from their roots, and residue of these causes inhibition of their seed germination and seedling growth (Yu, 2001; Kato-Noguchi et al., 2017). Further, various studies under the field or greenhouse or laboratory conditions have been conducted on the autotoxic effect of various plants such as sunflower (Irons & Burnside, 1982), Jet plant (*Crassula ovata*) (Chon et al., 2004), wheat (influence), and barley (Ben-Hammouda et al., 2002; Ashrafi et al., 2007). The study conducted by Irons & Burnside (1982) evaluated the effect of sunflower residues on the sunflower seeds germination. The results of this study revealed that the leaves were found more toxic than stem and root. Their secretion leads to the inhibition of seed germination as well as its growth. Further, the toxic effect was also reported on the plant height, wet weight, and dry weight and these parameters decreased significantly (Irons & Burnside, 1982). Similarly, an autotoxic effect of *Crassula ovata* was studied by Chon et al. (2004), these researchers also reported that the inhibition in the seed germination and seedling growth of *Crassula ovata*. Yu (2001) reported the allelopathic effect of *Triticum* (wheat) aquatic extracts on the growth of two other wheat varieties. Ben-Hammouda et al. (2002) reported the autotoxicity of *Hordeum vulgare* (barley) on the growth of other barley plants. The treatment results in the reduction of length and dry weight of vegetative and root system of recipient barley seedling as compared to the normal plant. From the same study, Ben-Hammouda et al. (2002) confirmed that the barley leaves were releasing more allelopathic compounds than the other plant parts. Stevenson, (1967) reported that the *Oryza sativa* (rice) plants released fatty acids as an allelopathic compound in the soil, which interfere with the growth of the successive rice plantation in the same field. This study also confirmed that the anaerobic conditions were suitable for releasing more fatty acids from residues by microbes (Stevenson, 1967).

Anagyris foetida (Stinking Bean Trefoil) is a small perennial tree with a height range between 1 to 2 meters. This tree is widely distributed in Iraq, Turkey, and other Mediterranean regions. Its stem is green, covered with white bristles. The leaves are composite contain 5-12 leaflets (GSPC, 2013). The leaves are lanceolate and the flowers are yellow, which blooms in March. Flower length can reach up to 5.2cm and after fertilization, it gives

horny fruit. This plant contains high concentrations of poisonous alkaloids such as cytisine anagyrine and aporphine (Carl, 2019). Alkaloids are a group of organic compounds which have one or more nitrogen atom associated with heterogeneous rings. So, the alkaloids do not share a specific chemical composition (Kittakop et al., 2014; Taher & Husain, 2021). It is found freely or in association with other organic compounds such as citric acid, tannic acid, tartaric acid, etc. (Russo et al., 2013). The plants containing a high concentration of alkaloids are the most important concerning their medicinal properties. The autotoxic characteristics are found in small quantities in all plant parts such as leaves, seeds, or fruits. Since, *A. foetida* contains a high concentration of alkaloids, it is important to know the autotoxic effect of this tree. Therefore, the current study was carried out to evaluate the autotoxic effect of various parts of *A. foetida*. Also, an attempt was undertaken to estimate the lethal concentration of the active compounds and eliminate the toxic substance in *A. foetida* through its residues.

2 Materials and Methods

The study was conducted at the Biology department laboratories of Education, Ibn-Alhaitham college, Baghdad University, Baghdad, Iraq from August 2014 to February 2015.

2.1 Sample collection

The *A. foetida* fruits were harvested during the ripening stage from the Hawiga area in Kirkuk governorate, Iraq. The fruits were dried in the shade and kept in a plastic bottle until used. The seeds were taken out, washed thoroughly 2 times with distilled water. The plants were uprooted with roots (vegetative system and root system). The plant material was washed thoroughly two times to remove unwanted parts. The roots were separated from the leaf and flowers. Roots, flowers, and leaves were dried in the sunlight and cut into small pieces followed by drying in the electric oven at 70°C for 3 days. Each sample was ground in the electric grinder separately. The samples were stored in the air-tight container till further use.

2.2 Aqueous extract Preparation

Three concentrations viz., 4, 8, and 12% of aquatic extracts were prepared from the root, flower, and leaves. The sample was thoroughly mixed for 15 min and filtered through the 3 layered quartz. The filtrate was again filtered with Whatman filter paper (No.1). The filtrate was further stored in a dark glass bottle at 5°C and protected from direct sunlight.

2.3 Potting Experiments

The 20 cm height pot was filled with a mixture of soil and pit moss (1:1). Two different experiments were designed. In the first

Table 1 Experimental design of aquatic extracts of *A. foetida*

Sr. No.	Aquatic extracts of <i>A. foetida</i>	Concentration (%)
1	No treatment	Control
2	Root aquatic extract	4 %
3	Root aquatic extract	8 %
4	Root aquatic extract	12 %
5	No treatment	Control
6	Vegetative aquatic extract	4 %
7	Vegetative aquatic extract	8 %
8	Vegetative aquatic extract	12 %
9	No treatment	Control
10	Flowering aquatic extract	4 %
11	Flowering aquatic extract	8 %
12	Flowering aquatic extract	12 %
13	No treatment	Control
14	Root +Leaf+ Flower aquatic extract	4 %
15	Root +Leaf+ Flower aquatic extract	8 %
16	Root +Leaf+ Flower aquatic extract	12 %

All the experiments were performed in triplicate for each treatment, 25 seeds were used.

experiment, the effect of various concentrations of root, flower, leaf, and mixture of all three aqueous extracts was evaluated on the *A. foetida* seed germination. For each treatment, 25 seeds were used and the experiment was repeated two times. While in the second experiment, the effect was evaluated after the seed germination, *i.e.* on the plant growth. In this experiment, 5 plantlets were used for each treatment and the experiment was repeated two times. The experimental design is depicted in Table 1.

2.4 Detection of Toxic Compounds in *A. foetida* by High Performance Liquid Chromatography

From each group around 1gm of leaves and seeds were collected. It was ground to a fine powder and dissolved in 10ml of distilled water. Potassium hydroxide (KOH) was used for maintaining pH 8.2. The sample was mixed thoroughly at room temperature for 10 minutes in the ultrasonic-wave bath. The sample was filtered to remove fibers. Then, the filtrate was concentrated by evaporating the solvent in liquid nitrogen until it becomes 0.5ml. The concentrated sample was evaluated for the presence of cytisine, aporphine, and anagyrine by a cylinder High Performance Liquid Chromatography. The compound quantification was carried out based on standard compounds analyzed under the same circumstances (Mandave et al., 2014).

2.5 Calculate Toxic Substance Concentration

The alkaloids concentration was calculated for leaves and seeds according to the equation given by Peivandi (1992).

Concentration (mg/ml) = Sample space/measuring space x Measuring concentration x Light factor for measuring space

2.6 Statistic Analysis

The results are represented as Mean $\pm$ SE (Standard error) for plant length. All the experiments were conducted in a Completely Randomized Design (C.R.D.) and analysis of variance (ANOVA) was calculated for the obtained data. The difference between the arithmetic means was tested at probability level 0.05 by use of the Duncan test polynomial (Poulin et al., 2018).

3 Results

In the present study, the autotoxic effect of roots, flowers, and leaves of *A. foetida* aqueous extract was evaluated against seed germination and plant growth. The concentrations of alkaloids were evaluated from the leaves and seeds of the plantlets after autotoxic treatment of various plant parts, *i.e.* roots, flower, and leaf. Table 2 represented the alkaloid concentrations.

Table 2 Calculation for alkaloids concentration

Sr.No.	Material name	Retention Time	Package area	Concentration (mg/ml)
1	Cytisine	1.22	59437	15mg/ml
2	Aporphine	2.39	87383	15mg/ml
3	Anagyrine	3.57	82563	15mg/ml

3.1 Effect of Various Aqueous Extracts on the Seed Germination and Seedling Growth

The percentage seed germination and length of the seedling (cm) are depicted in Table 3. Higher concentrations (12%) of the root, leaf, and flower aqueous extracts showed a negative effect on seed germination and seedling growth. The seeds treated with the lower concentration (4%) of the aqueous extract showed minimum mortality, *i.e.* maximum seed germination rate.

Table 3 Seed germination and seedling growth

Extracts type	Concentration	Seed germination (%)	Length of the seedling
Root	4%	12.32 ^d	6.81±1.32 ^c
	8%	6.31 ^c	3.00±0.98 ^b
	12%	5.15 ^b	3.75±1.00 ^b
	Control	22.89 ^a	23.89±3.72 ^a
Leaf	4%	14.29 ^b	7.02±3.82 ^b
	8%	5.01 ^c	3.79±4.71 ^c
	12%	2.76 ^d	1.27±0.98 ^d
	Control	22.25 ^a	22.89±1.47 ^a
Flower	4%	2.86 ^c	1.88±0.84 ^b
	8%	4.08 ^b	4.94±0.36 ^b
	12%	1.04 ^d	6.92±1.35 ^c
	Control	22.03 ^a	21.94±1.46 ^a
Root + leaf + flowers	4%	12.24 ^d	6.83±1.73 ^d
	8%	9.33 ^b	4.09±2.10 ^c
	12%	4.07 ^c	2.32±1.09 ^b
	Control	21.13 ^a	24.13±2.75 ^a

The results are represented as Mean ± SE (Standard error). Means sharing the same superscript letters under the same column do not differ significantly at P<0.05.

3.2 Estimation of *A. foetida* Alkaloids Concentration in the Leaves

Quantification of alkaloids (mg/ml) in leaves after treatment with various plant parts of *A. foetida* is depicted in table 4. The chemical analysis showed that the root aqueous extract of *A. foetida* at 4% concentration (7.15mg/ml) leads to a significant decrease in cytosine alkaloids as compared to the control treatment (29.25mg/ml). The flower and leaf aqueous extract at 12% concentration showed a significant decrease in the cytosine alkaloids 4.20mg/ml and 4.12mg/ml, respectively, as compared to the control treatment (29.25mg/ml). However, the mixture of three plant parts at 4% concentration showed a significant decrease of cytosine alkaloids (7.22mg/ml) as compared to the control treatment (29.25mg/ml). The aporphine alkaloid was found to be significantly decreased to 3.12, 3.21, 3.15, 3.34mg/ml in the plants treated with aqueous extract of root (4%), leaf (12%), flower (12%), and the mixture of three plant part @ 4% concentration respectively, as compared to the control treatment (13.12mg/ml). Similar trends were observed for the anagryne alkaloids content in the plants treated with root (6.23mg/ml), leaf (5.23mg/ml), flower (4.56mg/ml), and the mixture of three plant part (4.45mg/ml).

3.3 Estimation of *A. foetida* Alkaloids Concentration in the Seeds

Quantification of alkaloids (mg/ml) in seed after treatment with various plant parts of *A. foetida* is depicted in Table 5. *A. foetida* root aqueous extract (4%) showed a significant decrease in the cytosine alkaloids in the seeds as compared to the control treatment (11.14mg/ml). The flower and leaf aqueous extract (12%) of *A. foetida* showed a significant decrease to 3.32 and 2.56 mg/ml in cytosine alkaloids as compared to the control treatment (11.14mg/ml). The mixture of all three parts showed at 4% concentration showed a significant decrease (2.20mg/ml) in cytosine alkaloids as compared to the control treatment (11.14mg/ml). *A. foetida* root (4%), leaf (12%), flower (12%) and mixture of all three parts (4%) aqueous extract leads to significant decrease to 0.00, 0.56, 3.34 and 0.34mg/ml respectively in aporphine alkaloids as compared to the control treatment (3.34mg/ml).

A. foetida root aqueous extract at 4% concentration (2.11mg/ml) leads to a significant decrease in anagryne alkaloids as compared to the control treatment (8.70mg/ml). The flower and leaf aqueous extract (12% concentration) of *A. foetida* showed a significant

decrease in anagryne alkaloids to 0.22 and 2.62mg/ml as compared to the control treatment (8.70mg/ml). The mixture of all three parts showed at 4% concentration showed a significant decrease (2.31mg/ml) in anagryne alkaloids as compared to the control treatment (8.70mg/ml).

Table 4 Quantification of alkaloids (mg/ml) in leaves after treatment with various plant parts of *A. foetida*

Extracts type	Concentration	Cytisine	Aporphine	Anagryne
Root	4%	7.15 ^d	3.12 ^c	6.23 ^d
	8%	10.31 ^c	6.12 ^b	12.40 ^b
	12%	17.32 ^b	6.31 ^b	9.34 ^c
	Control	29.25 ^a	13.12 ^a	16.10 ^a
Leaf	4%	17.50 ^b	10.23 ^b	8.21 ^c
	8%	8.22 ^c	7.00 ^c	11.45 ^b
	12%	4.12 ^d	3.21 ^d	5.23 ^d
	Control	29.25 ^a	13.12 ^a	16.10 ^a
Flower	4%	7.22 ^c	7.22 ^b	7.23 ^c
	8%	11.30 ^b	7.21 ^b	11.00 ^b
	12%	4.20 ^d	3.15 ^c	4.56 ^d
	Control	29.25 ^a	13.12 ^a	16.10 ^a
Root +leaf + flowers	4%	7.22 ^d	3.34 ^d	4.45 ^d
	8%	22.50 ^b	6.22 ^c	11.28 ^b
	12%	12.67 ^c	10.26 ^b	8.05 ^c
	Control	29.25 ^a	13.12 ^a	16.10 ^a

Means sharing the same superscript letters under the same column do not differ significantly at P<0.05.

Table 5 Quantification of alkaloids(mg/ml) in seed after treatment with various plant parts of *A. foetida*

Extract type	Concentration	Cytisine	Aporphine	Anagryne
Root	4%	3.00 ^c	0 ^b	2.11 ^c
	8%	6.22 ^b	3.21 ^a	6.00 ^b
	12%	6.23 ^b	3.32 ^a	6.34 ^b
	Control	11.14 ^a	3.34 ^a	8.70 ^a
Vegetative	4%	5.95 ^b	3.22 ^a	6.00 ^b
	8%	6.22 ^b	2.99 ^{ab}	5.33 ^{bc}
	12%	3.32 ^c	1.00 ^b	2.62 ^c
	Control	11.14 ^a	3.34 ^a	8.70 ^a
Flowers	4%	9.00 ^b	3.00 ^a	4.50 ^b
	8%	5.27 ^c	3.23 ^a	4.51 ^b
	12%	2.56 ^d	0.22 ^b	1.20 ^c
	Control	11.14 ^a	3.34 ^a	8.70 ^a
Root +flowers + Leaf	4%	2.20 ^d	0.34 ^c	2.31 ^c
	8%	6.00 ^c	1.99 ^b	5.00 ^b
	12%	9.21 ^b	2.65 ^b	5.12 ^b
	Control	11.44 ^a	3.34 ^a	8.70 ^a

Means sharing the same superscript letters under the same column do not differ significantly at P<0.05.

4 Discussion

The effect of allelopathic plants was studied in detailed on various plants' growth and germination (Grainger et al., 2019; Malecore et al., 2019; Kattge et al., 2020; Oduor et al., 2020; Kong et al., 2021; Li et al., 2021). Further, Kong et al. (2021) studied the allelopathic effect of *Litsea pungens* essential oil on the seed germination rate and seedling growth of *Lolium perenne* and *Bidens pilosa*. These effects were due to the presence of around 17 terpenoids compounds in the *Litsea pungens* plant. Taher & Husain (2021) reported the allelopathic effect of *Triticum aestivum* aqueous extract on the division and growth of some wheat species. The current study also suggested that the alkaloids (anagyrine, cytisine, and aporphine) harmed the seed germination and plant growth of *A. foetida*.

4.1 Estimation of Alkaloids Concentration in the *Anagyris foetida* Leaves

The *A. foetida* residues at 4% concentration lead to a significant decrease in anagyrine alkaloids (4.45mg/ml) as compared to the control treatment (16.10mg/ml). Similarly, Kittakop et al. (2014) reported that the active substances in *A. foetida* leaves get reduced when the plant was treated with *Helianthus annuus* and white *Zea mays* residues. Many genetic and environmental factors regulate the movement of active compounds from the donor plant to the receptor plant. Similarly, genetic and environmental factors regulate the response of receptor plants towards the allelopathic compound (Russo et al., 2013). So, the effect of allelopathic compounds depends on the nature of the compound and its location in plants. Some compounds, especially phenolic acids may cause inhibition in the various activities of plants (Seigler, 2006).

4.2 Estimation of Alkaloids Concentration in the *Anagyris foetida* Seeds

The phenolic acids in the crop residues have electors effects and stimulation inhibition depending on influential concentration and environmental factors (Shamim, 2016). *A. foetida* contains many alkaloid compounds (Kittakop et al., 2014) that are from secondary allelopathic compounds which have an important role in germination inhibition (Stevenson, 1967). These compounds are generally toxic and have a special physiological effect. The poisonous allelopathic compounds hinder many physiological operations such as cell division and cell elongation inhibition which leads to the growth inhibition of plants (GSPC, 2013). Further, these compounds affect the plant's breathing, respiration, stomatal mechanism, and membrane permeability (Shamim, 2016). Where the allelopathic compound interfering with the cellular membrane in the plant that leads to a significant decrease in water absorption through roots. Yu (2001) suggested that this water deficiency might cause a reduction in plant growth and production.

Further, Zhang et al. (2021) reported four pathways through which the allelopathy effect was exerted by the plants. These pathways are leachate; residue; exudate and volatile pathways. In the present study, the leachate pathway was used by *A. foetida* to affect the plant growth and germination of other plants of the same species.

Conclusions

Aquatic extracts of flower, root, and leaf extract of *A. foetida* reduced the alkaloid content of *A. foetida*. Further, the recipient plant leaves showed a significant reduction in the alkaloid content, when the seeds were treated with *A. foetida* root (4%), flower (12%), leaf (12%), and all three parts mixture (4%) extracts. A similar pattern was observed for the *A. foetida* seed germination. Based on this study, it can be recommended that the aquatic extracts for *A. foetida* residues have a poisonous effect on plant growth. Its roots effectively secrete allelopathic compounds which inhibit *A. foetida* plant germination and growth. The allelopathic effect of plants can be studied in integrated management for the poisonous plant. Further, this study suggested that they should not repeat the *A. foetida* plantation in two successive years.

Conflicts of Interest: No

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