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ALLELOPATHIC POTENTIAL OF *RETAMA RAETAM* FORSSK ON SEED GERMINATION OF SOME MEDICINAL PLANTS

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ABSTRACT: *Laboratory experiments were conducted to determine the allelopathic effect of Retama raetam on seed germination of three medicinal plants (Lavandula stricta Del Achillea fragrantissima Forssk and Iphiona mucronata Forssk). The results indicated,that application of the different concentrations of shoot and root extracts of R. raetam in autumn and spring seasons seed germination of these three plants exhibited different responses, where the shoot extract was more effective than that of root to all the tested species. The preliminary phyotochemical screening of the extract of Retama raetam plant organs indicated that the shoot and root extracts of the two organs under study contain allelopathic compounds including Phenolic groups which can be used as natural herbicides or as a growth regulator.*

Key words: *Allelopathy, Retama raetam, phenolic compounds, phytochemical screening, and seed germination.*

INTRODUCTION

Allelopathy is a direct or indirect (harmful or beneficial) effects of a plant on another plant, through the release of chemicals from plant parts by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems (Rice, 1984 and Rizvi *et al.*, 1999). Allelopathy can make a significant contribution to competitive interactions (Reigosa *et al.*, 1999).

Recently, allelopathy has been exploited as a weed control strategy, alternative to the commercial herbicide dominated programs (Bhowmik, 2003).

Retama raetam Webb et Berth. Sub sp. *raetam* (RR), locally named as 'R'tm', is a spontaneous shrub legume belonging to *Leguminosae* family (*Fabaceae*). It is common to the North African and East Mediterranean region and the Sinai Peninsula w1x. The plant flowers from April to May; it is one of the most important plants in the East Mediterranean Deserts. (Boulos, 1999).

In semi-arid abandoned fields, the leguminous shrub *Retama sphaerocarpa* enhanced seedling survival of wild olive (*Olea europaea*) and

lentisc in south-facing slopes, whereas the opposite effect was seen in wild jujube (*Ziziphus lotus*) in both south- and north-facing slopes. It is likely that understory herbs and *Retama* roots interfered with the jujube plants, since survival was much higher in irrigated gaps between plants than under *Retama* (Padilla and Pugnaire 2006).

Alder and Chase (2007) studied the phytotoxicity of aqueous foliar extracts of sunn hemp (*Crotalaria juncea* L.), cowpea (*Vigna unguiculata* L.) and velvet bean (*Mucuna deeringiana* Bort) on goose grass (*Eleusine indica* L.) germination with 5% aqueous extracts of all cover crops (w/v fresh weight basis) was similar and greater than 75% of control. However, with the 10% extracts, goose grass germination was lowest with cowpea extract, intermediate with velvet bean extract, and highest with sunn hemp extract. Livid amaranth (*Amaranthus lividus* L.) germination declined to 50% with cowpea and sunn hemp extracts and even lower to 22% with velvet bean extract.

The magnitude of the impact of allelopathic compounds on plants depends on the type of compound, concentration and stability of that compound in the soil, and plant resistance and sensitivity to that compound. Among the types of chemical compounds identified as allelochemicals, cinnamic and benzoic acids derivatives, including ferulic and *p*-hydroxybenzoic acids, have frequently been mentioned in the literature (Einhellig, 1995).

Sparks (1999) stated that, "Phenolics comprise the largest group of secondary compounds in plants and are more often identified as allelopathic agents than all other compounds put together". Furthermore, phenolic compounds are water soluble and could easily be leached by rain, whereas leaves are still attached to the plant or, thereafter, from leaf litter.

In an excellent revision,Einhellig (2004) suggested that simple phenolic acids, affect the growth of plants and microbes through multiple physiological effects that confer on them a general toxicity. Owing to their aromatic nature, most of them interfere with processes where charge flux (electron or cations) is present, e.g. photosynthetic processes, electron deflectors, radical scavengers, inhibitors or competitors of the PSII system, ion membrane transport and permeability.

The objectives of the present work were to study the effect of application of different concentration of shoot and root extracts of *R. raetam* on the seed germination in the autumn and spring seasons.

MATERIALS AND METHODS

1. Materials

Germination experiments were conducted to study the allelopathic effect of *Retama raetam* on the seed germination of three medicinal plants in Wadi Hagul

The aerial parts and roots of *Retama raetam* were collected during the spring and autumn seasons of year 2007 from Wadi Hagul (East desert of Egypt). The plant material was air dried and ground to fine powder. Seeds of the tested species *Lavandula stricta Del Achillea fragrantissima* Forssk and *Iphiona mucronata* Forssk. were collected from killo 30 in wadi Hagul.

2.Preparation of total aqueous extract:

The dried samples were ground with a Wiley mill to fine powder. One hundred grams of dried shoot and root were extracted by soaking in 1L 70% methanol at 24 C for 24 h in a shaker. The extract was filtered through Whatman filter paper to remove the fiber debris then centrifuged at 5000 rpm for 15 min. The supernatant was subjected to vacuum and filtered again through Whatman filter paper, and the solvent was allowed to evaporate then completed to 1L by tap water (EC 0.65) to obtain the stock extract 10% (w/v).

3.Effects of aqueous extracts of *Retama raetam:*

The stock extract (10%W/V) was diluted appropriately with tap water to give the final concentrations of 1, 3, 5 and 10 percent (v/v). Tap water was the control. A Whatman No. 1 filter paper was placed in each 15-cm diameter glass Petridish. In each Petridish 50 seeds was used. Asuitable amount of diluted extract was pippetted to the filter paper. Three replicates were used for each treatment. Petridishes were left at room temperature conditions. Numbers of germinating seeds were counted every day. The emergence of the radical and the plumule was taken as a criterion for successful germination. Tests were terminated after three weeks when cumulative germination leveled off in all treatments. Data were transformed to percent of control.

4.Phytochemical screening:

The aerial parts and roots of *Retama raetam* extracts *were* tested qualitatively for the presence of chemical constituents according to (Sofowora A.1993, Trease G.E., and Evans, W.C. 1989 and Harborne 1973).

4.1.Test for alkaloids.

About 0.5 g of extract was diluted to 10 ml with acid alcohol, boiled Then filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia,then 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids. .(Trease G.E., and Evans, W.C. 1989)

4.2.Test for flavonoids.

Three methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow coloration that disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicates the presence of flavonoids. (Sofowora A.1993)

4.3.Test for saponins.

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigourously.and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigourously after which it was observed for the formation of an emulsion. (Harborne 1973).

4.4.Test for tannins.

About 0.5 g of the extract was boiled in 10 ml of water in a test, tube, Then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.(Trease G.E., and Evans, W.C. 1989)

4.5.Test for terpenoids (Salkowski test).

To 0.5 g of each extract was added 2 ml of chloroform. Concentrated H_2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids (Harborne 1973).

4.6.Test for coumarins:

In a test tube one g of plant sample was placed and covered with filter paper moistened with dil. NaOH , then heated on a water bath for a few minutes. The filter paper was examined under UV light, yellow fluorescence is indicative for the presence of coumarins(Harborne 1973).

5.Total Phenolics determination:

Total phenolics content of the shoot and root extracts of *Retama raetam* were executed with the Folin-Ciocalteu method. Two hundred micro liters of sample and 0.5 mL of Folin-Ciocalteu reagents (Merck) were added to 10 mL of distilled water in a 25 mL flask. After 3 min, 1 mL of saturated Na_2CO_3 was added, and the volume was made up to 25 mL. The samples were left for 1 h in the dark, then the absorbance was measured at 725 nm against a blank. A

calibration curve with caffeic acid was established, where 25-200 µg of caffeic acid in 100 µL of distilled water was used instead of the sample, and the total phenolic content was expressed in milligrams of caffeic acid equivalent per gram of extract (Liu *et al.*, 2002).

6.Statistical analysis:

Data are expressed as mean ± SD and analyzed by SPSS version 10.0 one way ANOVA followed by the use of LSD test for the differences between individual means. The 0.05 and 0.01 levels were selected as apoint of minimum statistical significance in every comparison.(snedecor and Cochran 1967)

RESULTS AND DISCUSSION

1.Effect of different concentrations of total aqueous Shoot extract of *R. raetam* in the autumn and spring seasons.

The effect of different concentrations of the aqueous shoot extract of *R. raetam* was tested for the seed germination of three species in Petridishes. Results showed that the aqueous extract of shoot inhibited seed germination percentage of *Iphiona mucronata* to zero while increased that of *Lavandula stricta* which reached 100% at 10% concentration of *R. raetam* shoot extract. In contrast, there was no effect of any aqueous extract concentration on the seed germination of *Achillea fragrantissima* (Fig 1). This findings are quite in agreement with Callaway and Aschehoug (2000) who mentioned that allelopathic plants may not have a strong allelopathic toxicity to their neighbors species (i:e, neighboring species have evolved resistance mechanism), whereas the same allelopathic plants may have a strong phytotoxicity to neighboring species in the same community. The allelopathic effects of plants could be relatively specific; for example, whereas the grass *Deschampsia flexuosa* was severely inhibited by *Petridium aquilinum* litter. A similar grass Holcus was not affected (Willis 2007).

The effect of different concentrations of the aqueous shoot extract of *R. raetam* in the spring season was tested for seed germination of three species in Petridishes. Results showed that the aqueous extract of shoot inhibited seed germination percentage of *Iphiona mucronata* while increased that of *Lavandula stricta* which reached 70% at 10% concentration of *R. raetam* shoot extract. There was no effect of any aqueous extract concentration on the seed germination of *Achillea fragrantissima* in both autumn and spring seasons. In contrast with the results obtained in the autumn season we found that The aqueous extract of shoot in spring season was the least inhibitory to the *Iphiona mucronata* The highest seed germination percentage was obtained with *Lavandula stricta* in autumn season that reach 100% in autumn season and 70% in spring season (Fig.2). These results in compatible with

Higashinakasu *et al.*, (2004) in which they reported that chemical substances that released from plant organs into the neighboring environment stimulate or suppress the development and growth of other plants.

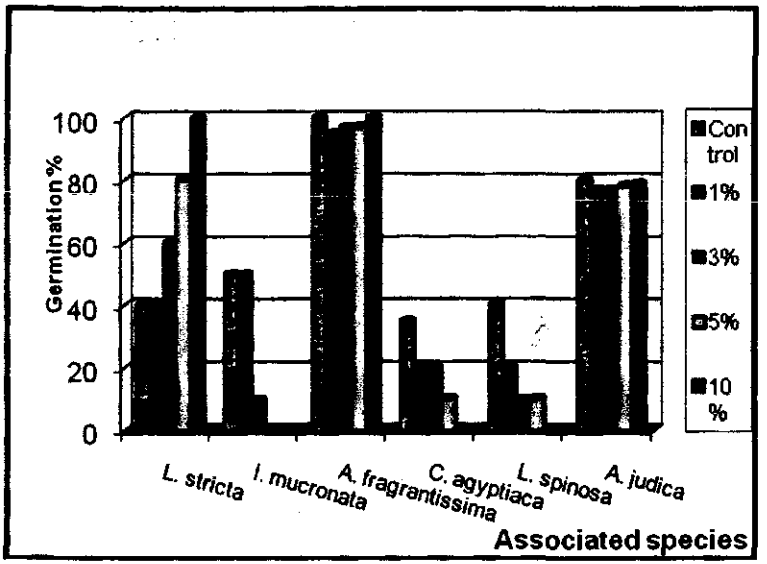

Fig. (1): Effect of different conc. of total a queous shoot extract of *R. raetam* Forssk in the autumn season .

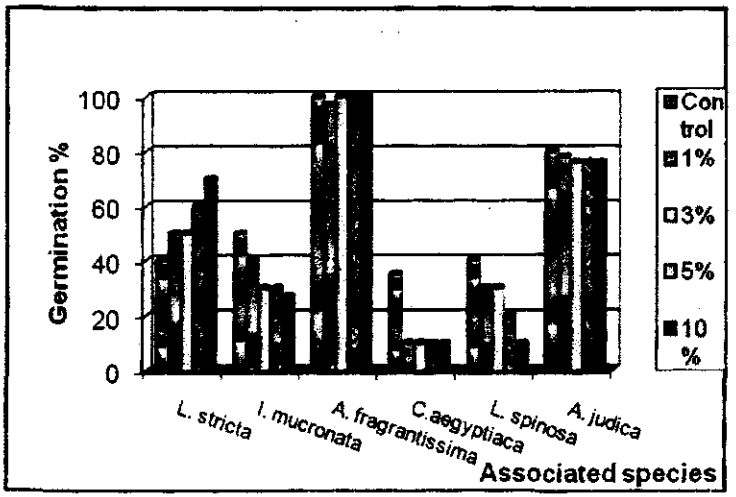

Fig. (2): Effect of different conc. of total a queous shoot extract of *R. raetam* Forssk in the spring season .

2. Effect of different concentrations of total aqueous root extract of *Retama raetam* in the autumn and spring seasons.

The effect of different concentrations of the aqueous root extract of *R. raetam* in the autumn season was tested on seed germination of three species in Petridishes. The seed germination percentage of *Iphiona mucronata, was* decreased by increasing concentration of the root extract to reach (22%) at 10% concentration root extract,while that of *Lavandula stricta* was accelerated by increasing concentration of this extract to reach the highest germination percentage (65%) at the highest concentration of *R.raetam* root extract (10%) and the lowest percentage (30%) at the lowest concentration (1%) (Fig 3).

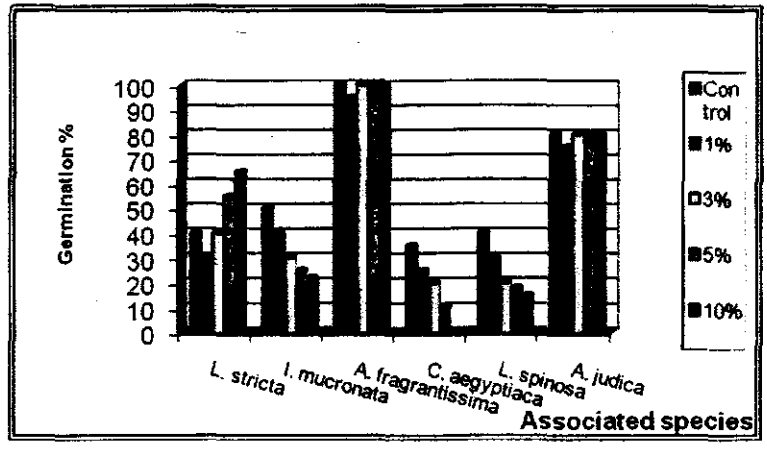

Fig. (3): Effect of different conc. of total a queous root extract of *R. raetam* Forssk in the autumn season .

The ability of root exudates to stimulate germination is of wide occurrence in the plant kingdom (Khalid *et al.* 2002)..These results are in agreement with Rizvi *et al.*, (1999) who reported that plant parts have been shown to reduce the yield of some plants but increase the yield of other plants (selective activity).and also in agreement with Higashinakasu *et al.*, (2004).However *Achillea fragrantissima*, showed no response to this extract.

The effect of different concentrations of the aqueous root extract of *R. raetam* in the spring season was tested on three species in Petridishes. In contrast with the results obtained in the autumn season we found that seed germination of *Iphiona mucronata* also decreased with increasing concentration of root extract of *R. raetam* in the spring season but the germination percentage decreased in autumn season more than in the spring season. In *Lavandula stricta* also we found that the seed germination percentage increased with increasing concentration of root extract that reach

to 60% at the highest concentration of this extract. *Achillea fragrantissima* also didn't affected by different concentrations of this extract (Fig 4).

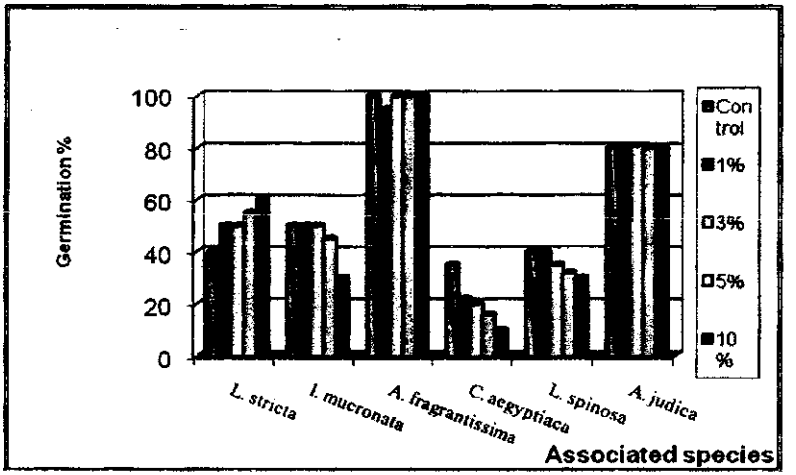

Fig. (4): Effect of different conc. of total a queous root extract of *R. raetam* Forssk in the spring season .

From the above results it appear that by applying the different concentrations of shoot and root extracts of *R. raetam* in autumn and spring seasons seed germination of tested plants exhibited different responses Although seed germination of *lavandula stricta* was increased by increasing concentration of shoot and root extracts in autumn and spring seasons the increase was higher in autumn than in spring and with shoot extract than root extract. On the other hand seed germination of *Iphiona mucronata* decreased by increasing concentration of shoot and root extracts in autumn and spring seasons and the decrease was higher in autumn than in spring and with shoot extract than root extract These results are in harmony with El Khtib and Abdelaah (1998) who reported that *Zilla spinosa* extracts exhibited different inhibitory effect on the seed germination and seedling growth of its associate species. Shoot reduced the percentage germination and seedling length of different test species more than root. In semi-arid abandoned fields, the leguminous shrub *Retama sphaerocarpa* enhanced seedling survival of wild olive (*Olea europaea*) in south-facing slopes, whereas the opposite effect has been observed in wild jujube (*Ziziphus lotus*) (Padilla and Pugnaire 2006).

Seed germination of *Achillea fragrantissima* did not show any effect by applying different concentrations of shoot and root extracts in autumn and spring seasons These results are in agreement with Inderjit and Duke (2003) who found that some plants have the ability to avoid the toxicity of allelochemicals produced by the neighboring plants through detoxification

mechanisms such as carbohydrate conjugation, sequestration or secretion, and oxidation of phototoxic compounds.

3. Phytochemical Screening of *Retama raetam* Forssk.

From Table (1) it can be concluded that: *R. raetam* plant contain allelopathic compounds in which shoot contains alkaloid, flavonoids, tannins, unsaturated sterols and/or triterpens coumarins , and traces of saponins whereas *R. raetam* root contains flavonoids, unsaturated sterols and/or triterpens and traces of alkaloid, saponins, tannins and coumarins

Table (1): Phytochemical Screening of *Retama raetam* Forssk.

Test for	Shoot	Root
Alkaloids	+	±
Flavonoids	+	+
Saponins	±	±
Tannins	+	±
Unsaturated sterols and/or triterpens	+	+
Coumarins	+	±

(+): present, (±) trace.

3.1.Phenolic contents of *Retama raetam:*

The shoot and root extracts of *Retama raetam* contain aconsiderable amount of phenolic compounds and the total phenolics of shoot was more than those of root and the total phenolics in autumn season was more than that in the spring season.(Table 2)

Table (2): Total phenolics (mg/g) of *Retama raetam* extracts.

Plant extract	autumn	spring
Shoot extract	198±3.8	193±4.45
Root extract	105±2.75	98±2.35

From these results it appears that the allelopathic compounds responsible for the allelopathic effects of aqueous extract of *Retama raetam* plants may be phenolic compounds. These results were in general agreement with those of Sparks (1999) who stated that: Phenolics comprise the largest group of secondary compounds in plants and are more often identified as allelopathic agents than all other compounds put together. Furthermore, phenolic compounds are water soluble and could easily be leached by rain while leaves are still attached to the plant or, thereafter, from leaf litter.

Allelopathic effects on plants may be caused by many types of secondary metabolites, including phenolic compounds (Kuiters and Sarink, 1986). Phenolic acids in the literature on allelopathy are often mentioned as putative allelochemicals and are perhaps the most commonly investigated compounds among potential allelochemicals. These compounds are found in a wide range of soils or plants and their phytotoxic potential against various test plants has been demonstrated under controlled conditions (Rice, 1984). Phenolic compounds are among the most abundant groups of secondary metabolites in plants, which bear hydroxylated aromatic rings including simple phenols, phenolic acids, phenylpropanoids, coumarins, quinones, flavonoids, tannins and other miscellaneous phenols (Harborne, 1980). All these compounds are known to be of significance in allelopathy (Inderjit, 1996).

From the above-mentioned results, it could be concluded that *Retama raetam* had apositive allelopathic effect on *Lavandula stricta* and negative allelopathic effect on *Iphiona mucronata* but with no effect on *Achilla fragrantissima* such plant contains allelopathic compounds and the allelochemicals responsible for this action may be Phenolic compounds. Because of this action such plant can be used as anatural herbicide or as a growth regulator.

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تاثير التضاد الكيميائى الكامن لنبات الرتم على انبات بذور بعض النباتات الطبية

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الملخص العربي

تمت هذه الدراسة لقياس تأثير الإبعاد التضادى الكيميائى لنبات الرتم (العائلة البقولية) على إنبات بذور ثلاث نباتات طبية هي الدكتاى والجصوم والدفرة وقد أوضحت الدراسة أن النباتات المختبرة تراوحت حساسيتها لتأثير المستخلص المائي للمجموع الخضري والمجموع الجذري في موسمي الخريف والربيع وان المستخلص المائي للمجموع الخضري كان أكثر تأثيرا من المستخلص المائي للمجموع الجذري على إنبات بذور الأنواع المختبرة. وقد أظهرت نتائج البحث الكيميائي لنبات الرتم احتواء مجموعه الخضري والجذري على مواد اليلوبائية والتي يمكن أن تستخدم كمضاد للحشائش أو كمنظم للنمو وان المركبات الفينولية ربما تكون هي المسئولة عن التأثير الاليلوبائى لهذا النبات.