Journal of Medicinal Plants and By-products (2018) 1: 75-81

Original Article

Allelopathic Potentials of Medicinal Plant, *Vinca rosea* L.

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Article History: Received: 30 November 2015 /Accepted in revised form: 20 January 2018

Abstract

Allelopathy offers potential for weed control through the production and release of allelochemicals from leaves, flowers, seeds, stems and roots. In this research, allelopathy potentials of ethanolic and aqueous extracts of *Vinca rosea* L. leaves were evaluated. Three extract concentrations, including 2.5, 5 and 10% (w/v) of both aqueous and ethanolic were prepared from a 10% w/v stock solution in a completely randomized design with three replications. Distilled water was used as negative control. The inhibitory effects of *V. rosea* extracts on seed germination, hypocotyl (the part of the stem of an embryo plant) and radicle (the part of a plant embryo that develops into the primary root) length and seedlings growth of five plants were tested. The plants were cucumber (*Cucumis sativus*), redroot amaranth (*Amaranthus retroflexus*), garden cress (*Lepidium sativum*), scale cereal (*Secale montanum*) and desphia herb (*Descurainia sophia)*. Results showed that the 10% aqueous extracts on the *A. retroflexus, D. sophia* and *L. sativum* and 10% ethanolic extracts on the five tested plants inhibited seed germination completely. The 10% aqueous extract inhibited the hypocotyls lenght of *L. sativum*, *A. retroflexus* and *D. sophia* completely. The 10% aqueous and ethanolic extract of *V. rosea* inhibited the radicle lenght of *L. sativum*, *A. retroflexus* and *D. sophia* completely. The inhibitory effects of the *V. rosea* extract may be related to the presence of allelochemicals, including alkaloids, flavonoids and phenolic acids. The use of allelopathic materials into agricultural management may reduce the use of pesticides and lessen environmental side effects.

Keywords: Allelopathy, Ethanolic and aqueous extracts, Medicinal plant, *Vinca rosea*

Introduction

Allelopathy is a biological phenomenon by which an organism produces and excretes one or more chemical substances that influence the growth, survival, and reproduction of other organisms. These substances are known as allelochemicals and can have beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target organisms [1]. Allelopathy is an important mechanism of plants interference mediated by the addition of plant-produced phytotoxins to the environment and is considered as a competitive strategy on the part of plants [2]. Allelochemicals are produced by plants as the end products or intermediate. These metabolites are present in the stems, leaves, roots, flowers, inflorescences, fruits and seeds [3]. The release of these chemical compounds into the environment acts on other organisms such as plants, including weeds, animals and microorganisms to either inhibit or stimulate activities [4]. There is increasing evidence that these plant chemicals can suppress germination and growth of different weed species [5-8]. Worldwide, enormous amounts of chemical herbicides are used to control these weeds. However, synthetic herbicides are often toxic and cause environmental pollution [9,10]. Moreover, overuse of artificial herbicides has led to the development of weed biotypes with herbicide resistance [9]. In agriculture, there is a worldwide effort to reduce the amount of chemicals used in crop production through modern biological and ecological methods. One of the possible solutions is the use of allelopathy to cop the negative chemical

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interactions among plants [11]. The importance of allelopathy in the natural weed control and crop productivity is now highly recognized [12]. In recent years, medicinal plants have been increasingly explored for their allelopathic potentials [13]. Medicinal plants may contain bioactive compounds such as ferulic, coumaric, vanillic, caffeic and chlorogenic acid that possess inhibitory activity [14,15]. When evaluated the allelopathic effects of the aqueous extracts of *Rheum emodi*, *Saussaurea lappa* and *Potentilla fulgens* on some traditional food crops; the germination of all crops seeds was significantly reduced by *S. lappa* and *P. fulgens* extracts [4].

Vinca rosea Linn, commonly known as the Madagascar perivenkle is one of the most important medicinal-ornamental plants that belongs to Apocynaceae family [16,17]. This plant is a perennial shrub with green color, simple entire, petiolate leaves and violet pink-white or carmine red color flowers [18]. It contains more than 100 alkaloids including vincristine, catharanthine, anhydrovinblastine, ajmalicine, strictosidine, vinblastine, resrepine and serpenthine [19].

In this research, the allelopathy potentials of ethanolic and aqueous extracts of *V. rosea* leaves were studied. The inhibitory effects of *V. rosea* extracts on seed germination, hypocotyl (the part of the stem of an embryo plant) and radicle (the part of a plant embryo that develops into the primary root) length and seedlings growth of five plants including cucumber, redroot amaranth, garden cress, scale cereal and desphia herb have been considered.

Material and Methods

Plant Material and Extraction

Fresh leaves of *V. rosea* were collected from the Eram Botanical Garden, Shiraz, Iran. Leaves were washed several times with distilled waterand dired in shade. The dried leaves were powdered in a grinder and soaked (20 g) in 200 mL (1:10 W/V) ethanol and water seperately and shaked for 24 hr. The extracts were filtered through Whatman No.1 filter paper. The remaining residues were re-extracted twice and the extracts were pooled. The solvent in ethanolic exteract was removed under vacuum at 40°C using a rotary vacuum evaporator. The seeds of cucumber (*Cucumis sativus*), redroot amaranth (*Amaranthus retroflexus*), garden cress (*Lepidium sativum*), secale cereal (*Secale montanum*) and desphia herb (*Descurainia sophia*) were chosen for this study. The experimental design was a completely randomized design with three replications.

Bioassay

In order to study the allelopathic effects of the *V.rosea*, different concentrations (2.5, 5 and 10%) of the original leaf extracts were prapred. Twenty seeds of each weed were surface sterilized by 5% sodium hypochloride for 5 minutes and after several rinsing with distilled water they were placed on sterilized filter papers inside petri-dishes and three mL of the leaves extract were added. Distilled water was used as control. Petri dishes were placed in the light (350 µmol m^{-2} s^{-1}) at 25°C for 10 days. The seeds were monitored daily and distilled water was used to make up for the water evaporated from the petri dishes. At the end of the 10th day, percent of seeds germination,seeds radicles and hypocotyle lengths were determined. Seeds with at least 2 mm radicles length were considered to have germinated.

Statistical Analysis

The experimental design was a completely randomized design with three replications for each treatment. Data were analyzed using SPSS v. 17.0 and mean comparisons were made following the LSD test at $P \leq 0.01$.

Results

Variance analysis of the *V. rosea* leaves extract showed a significant ($P \leq 0.01$) decrease in the rates of seeds germination and seedlings growth of five studied plant including cucumber, redroot amaranth, garden cress, scale cereal and desphia herb (Table 1). The mean comparisons are shown by letters in the figure 1. The different letters show significant differences ($P \leq 0.01$).

Seed Germination

The germination rates of the five tested seeds responded differently to concentrations of aqueous and ethanolic extracts. In all control groups, the germination percentage was 80-100%. The 3 mm root lenght and was used for germination index. Aqueous extract (5 and 10%) of *V. rosea* inhibited the seed germination of all tested plants except *C. sativus* significantly ($P \leq 0.01$). The 2.5% aqueous leaves extracts inhibited the seed germination of *A.*

Retroflexus, *D. sophia* and *L. sativum* significantly ($P \leq 0.01$). The 2.5% ethanolic extract inhibited all plants except *C. sativus*, but the 5 and 10% ethanolic extracts inhibitited germination significantly ($P \leq 0.01$) of all five tested plants. The 5% ethanolic extract inhibited the germination of *A. retroflexus* completely. The 10% aqueous extracts on the *A. retroflexus*, *D. sophia* and *L. sativum* and 10% ethanolic extracts on the five tested plants inhibited seed germination completely (Fig1).

Hypocotyl Lenght

All concentration of ethanolic and aqueous extracts except 2.5% aqueous extracts on *S. Montanum*, had negative effect on the hypocotyl length significantly ($P \leq 0.01$). The 10% aqueous extract inhibited the hypocotyls lenght of *L. sativum*, *A. retroflexus* and *D. sophia* completely. The 5% ethanolic extract inhibited the hypocotyls lenght of *A. Retroflexus* completely. The 10% ethanolic extract inhibited the hypocotyls lenght of all five tested plants completely (Fig1). The seeds of *S. montanum* did not germinate (3 mm root lenght for germination index) with 10% ethanolic extract of *V. rosea* but it had the hypocotyl with 1mm lenght.

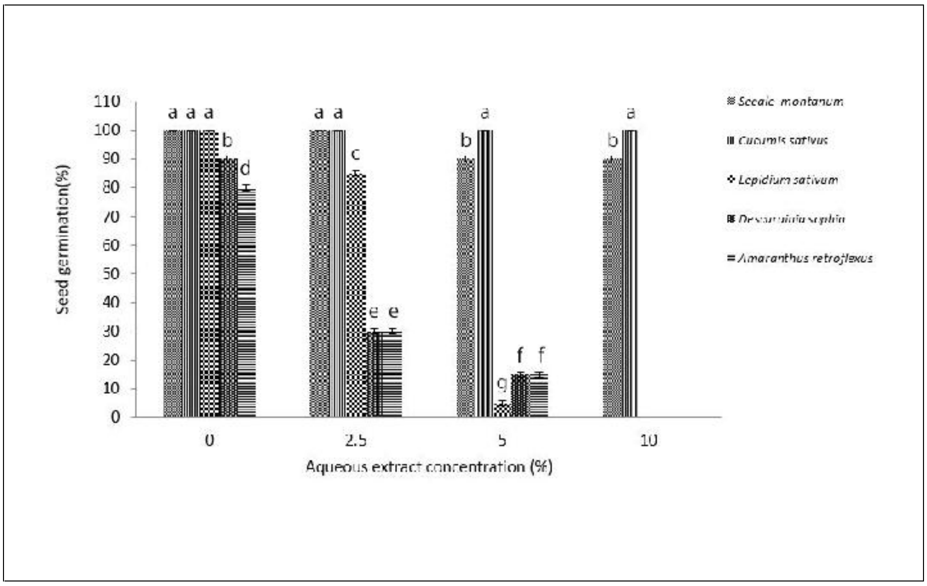
Radicle Lenght

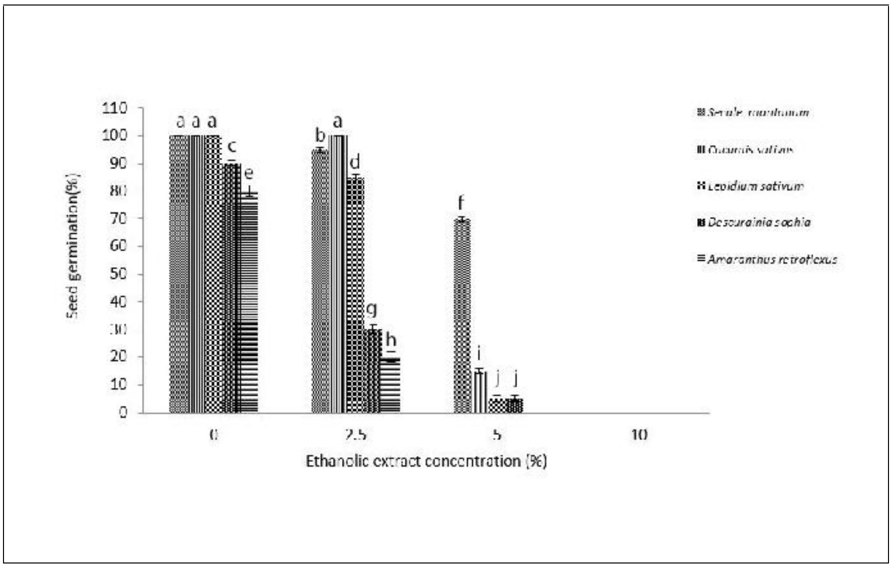
All concentration of ethanolic and aqueous extracts had negative effect on the radicle length of five tested plants significantly ($P \leq 0.01$). The 10% aqueous and ethanolic extract of *Vinca rosea* L. inhibited the radicle lenght of *L. sativum*, *A. retroflexus* and *D. sophia* completely (Fig1). The 10% ethanolic extract inhibited the germination of five tested plants completely but the *C. sativus* and *S. montanum* had 1.5 mm radicle under 10% ethanolic extract which was below the root lenght (3 mm) for germination index.

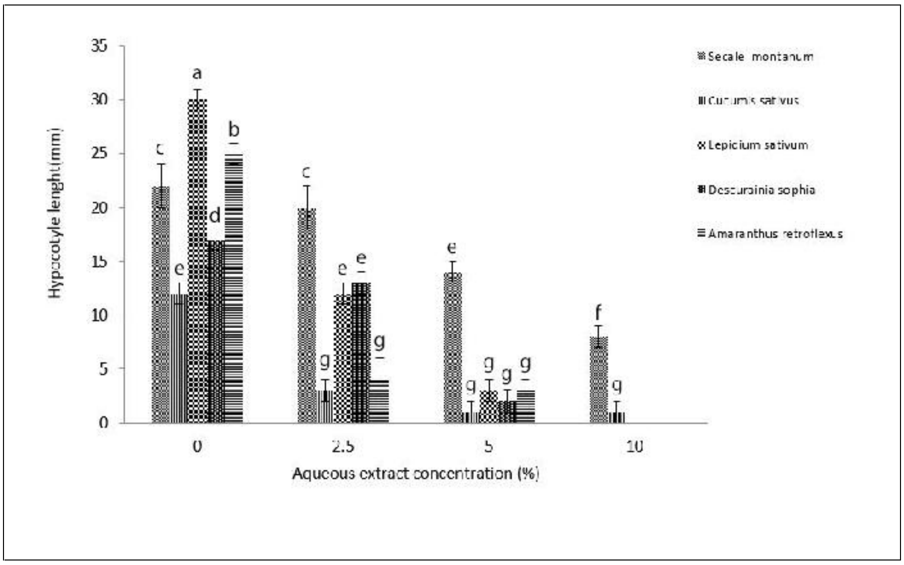
Table 1 Analysis of variance of *Vinca rosea* L. extracts on seed germination and seedlings growth of five tested plants

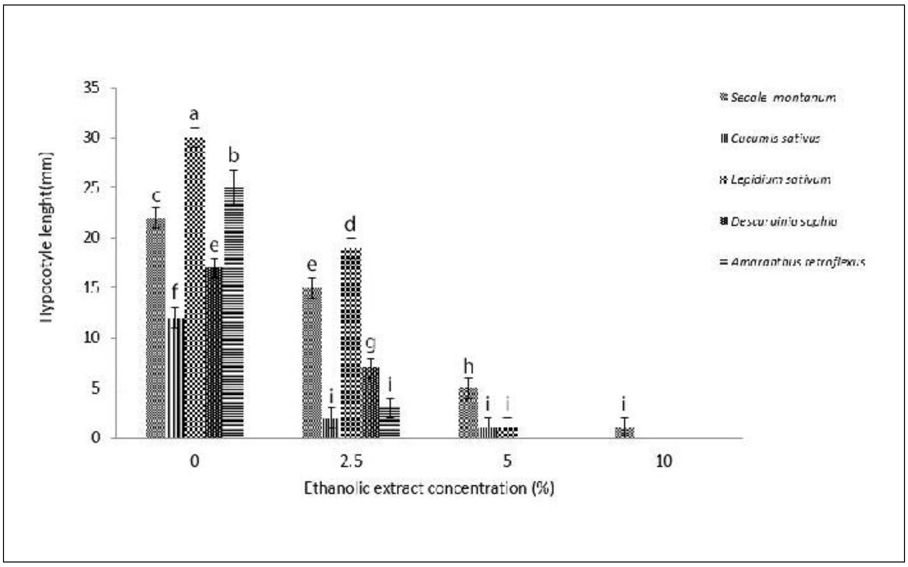
Source of Variation	df	Mean squares		
		Germination (%)	Hypocotyl length	Radicle length
Aqueous extract	19	5490.83**	234.06**	1250.60**
Ethanolic extract	19	5848.33**	246.93**	1222.22**
Error	40	1.634	1.517	1.950

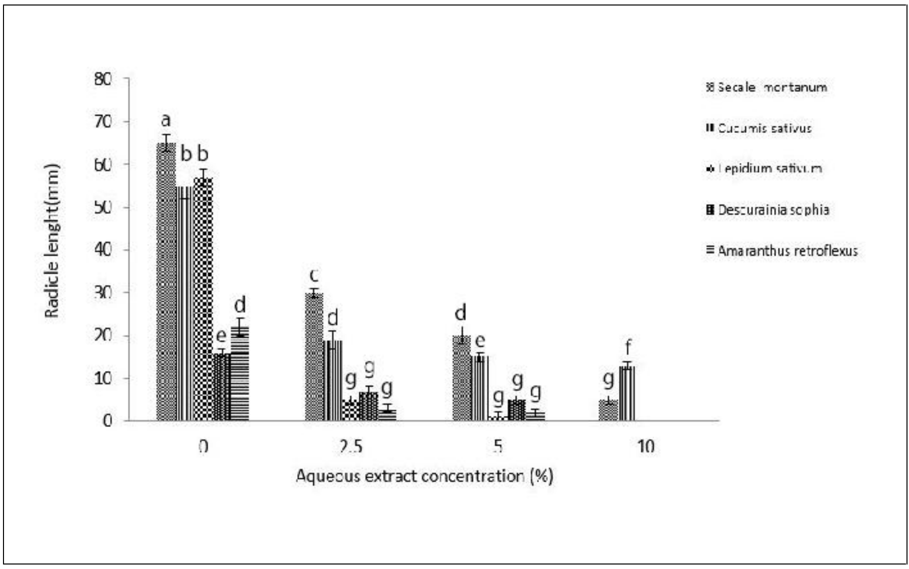
**: Significant at 0.01 probability level




Seed germination(%)
Ethanolic extract concentration (%)
Secale montanum
Cucumis sativus
Lepidium sativum
Descurainia sophia
Amaranthus retroflexus


Hypocotyle lenght(mm)
Aqueous extract concentration (%)
Secale montanum
Cucumis sativus
Lepidium sativum
Descurainia sophia
Amaranthus retroflexus


Hypocotyle lenght(mm)
Ethanolic extract concentration (%)
Secale montanum
Cucumis sativus
Lepidium sativum
Descurainia sophia
Amaranthus retroflexus



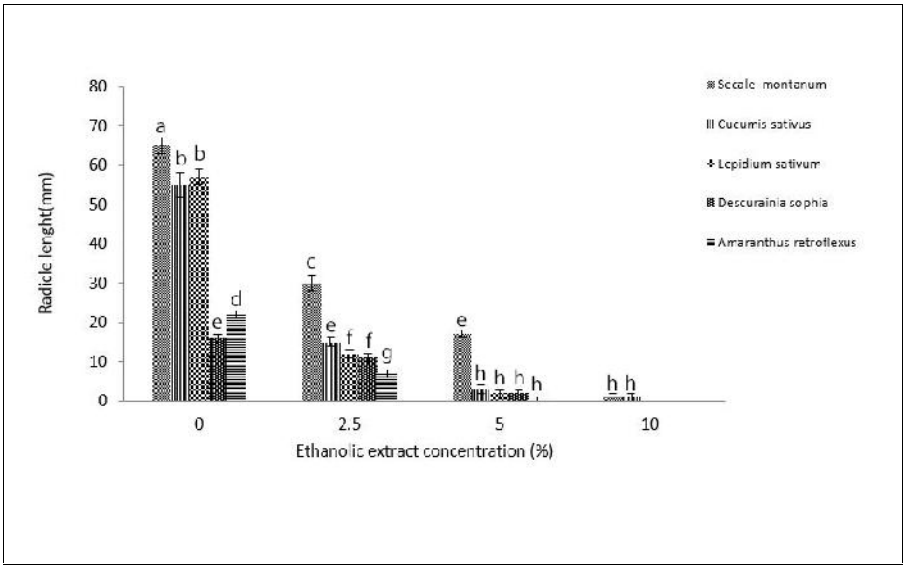


Fig. 1 Effect of different concentrations of *Vinca rosea* L. leaf aqueous and ethanolic extract on seed germination, hypocotyl and radicle lenght of cucumber (*C. sativus*), redroot amaranth (*A. retroflexus*), gareden cress (*L. sativum*), secale cereal (*S. montanum*) and desphia herb (*D. Sophia*). Different letters show significant differences ($P \leq 0.01$).

Discussion

Certain plant species or their residues inhibit the development of particular species selectively. This differential sensitivity has been observed in the field, greenhouse and laboratory experiments with plants residues, plants extracts and purified allelochemicals [20,21]. Allelopathy plays a major role in agricultural management such as weed control, crop protection, and crop re-establishment [22]. Most assessments of allelopathy involve bioassays of plant or soil extracts, leachates, fractions, and residues based on seed germination and seedling growth in laboratory and greenhouse experiments [22]. In this study, basic study on the allelopathic potential of different concentrations of ethanolic and aqueous extracts of *V. rosea* leaves showed that this medicinal plant exhibited a significant inhibitory effect on the seed germination and seedling lengths of all five examined plants, especially at high concentration. The inhibitory effects of ethanolic extracts on hypocotyl and radicle lengths were higher than those of aqueous extracts. This maybe is related to better extraction potential of ethanol. The results showed that the radicle lengths were more decreased than hypocotyl length. Figure 1 shows that the 10% concentration of *V. rosea* leaf ethanolic and aqueous extracts can be used as natural herbicide against amaranthus weeds and desphia herbs. These result showed that the *A. retroflexus*, *D. sophia* and *L. sativum* were more sensitive to *V. rosea* leaf ethanolic and aqueous extracts than *C. sativus* and *S. montanum*, so *V. rosea* extract can be used as natural herbicides for these weeds because inhibit completely the seed germination and seedling growth. Plant growth may be stimulated below the allelopathic threshold, but severe growth reductions may be observed above the threshold concentration depending upon the sensitivity of the receiving species. Generally germination is less sensitive than is seedling growth, especially root growth [22]. In our research, the more reduction of seed germination was observed. Delayed seed germination and slow root growth due to the extracts could be confounded with osmotic effects on rate of imbibition, delayed initiation of germination, and especially cell elongation; the main factor that affects root growth before and after the tip penetrates the seed coat [22]. The incorporation of allelopathic substances into agricultural management may reduce the use of pesticides and lessen environmental deterioration [22]. The inhibitory effects of the *V. rosea* extract on seed germination and seedling growth of the five tested plants may be related to the presence of allelochemicals, including alkaloids, flavonoids and phenolic acids. Furthermore, toxicity might be due to a synergistic effect rather than the effect of any one compound or class of secondary metabolite [23]. Cytotoxicity can be regarded as the quality of being toxic to cells. Some secondary metabolites produced by some plants possess cytotoxic properties. For example, *V. rosea* possesses secondary metabolites such as vinblastine and vincristine, which are known for their cytotoxic properties [24]. Allelopathic phytochemicals act by inhibiting the germination of plants thereby disrupting the cell division, interfering with the mechanism of energy transfer and limiting water and nutrient uptake [25]. The lower water availability for seed germination due to binding water by compounds present in an extract might play an effective role in reducing seed germination [26].

Acknowledgments

We would like to acknowledge Mohsen Hamedpour-Darabi for English editing of the manuscript and Marjan Mohsenzadeh for excel and Photoshop edition.

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