Allelopathic Effect of Some Medicinal Plant Substances on Seed Germination of *Amaranthus retroflexus* and *Portulaca oleraceae*

M. Azizi
Faculty of Agricultural
Ferdowsi University
Mashhad
Iran

Y. Fuji
National Institute for Agro-Environmental Sciences
Tsukuba, Ibaraki
Japan

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Abstract

The world consumption of medicinal plants as pharmaceuticals, cosmetics and as a food supplement for the improvement of human welfare is increasing day by day. In agriculture there is a world-wide effort to reduce the amount of chemicals used in production by introducing modern biological and ecological methods. One of the possible solutions is allelopathy, the utilization of the chemical interaction between plants. In this research we studied the effect of several concentrations of a hydro-alcoholic extract of St. John's wort (*Hypericum perforatum)* and sage (*Salvia officinalis)* (undiluted, 1/5, 1/10 and 1/20 diluted) and different concentrations of Eucalyptus oil (*Eucalyptus globulus)*(0.1, 0.2, 0.5 and 1% v/v) on seed germination percent and rate for two weeds, *Amaranthus retroflexus* and *Portulaca oleraceae*. The statistical design was a randomized complete block design with 4 replicates. In the second experiment, we studied the effects of different concentrations of Black zira (*Bunium pesicum)* on lettuce and radish seed germination. Results showed that an undiluted extract of St. John's wort and sage had a significant inhibitory effect on seed germination percent and germination rate for *Amaranthus retroflexus,* but not for *Portulaca oleraceae*. Eucalyptus essential oils had a strong inhibitory effect on the germination of both weed species at 0.7% (v/v). *Bunium pesicum* essential oils also had an inhibitory effect on lettuce and radish seeds. Lettuce seeds were more sensitive than radish to these essential oils. Lettuce seed germination was completely stopped at 0.2% (v/v), but radish required a much higher concentration (1% v/v). The strong inhibitory effects of *Eucalyptus* and Black Zira essential oils may give promising results in the organic culture of plants.

INTRODUCTION

Allelopathy is defined as direct or indirect interaction, whereby chemicals and the metabolites released by one plant or organism, influence the physiological processes of another neighbouring plant or organism (Rice, 1984; Challa and Ravindra, 1998). Allelopathy is one of the most controversial ecological interactions (Harper, 1977; Williamson, 1990). In general, the chemical interactions that occur among living organisms including plants, insects and microorganisms are called allelopathy, and organic compounds involved in allelopathy are called allelochemicals. The release of allelochemicals from plants occurs by volatilization, leaching from leaves, exudation from roots and degradation of dead plant tissues. All plant parts have been shown to contain allelochemicals, but leaves and roots are the most important source (Whittaker and Feeny, 1971; Rice, 1979; Rizvi and Rizvi, 1992). The release of chemical compounds into the environment acts on other organisms such as weeds, plants, animals and microorganisms to either inhibit or stimulate activity (Fuji et al., 2003). Fuji et al. (1991) state that medicinal plants show relatively strong allelopathic activity. Sometimes a single chemical produced by one organism or plant is harmful to another but beneficial to a third organism or plant (Whittaker and Feeny, 1971; Rice, 1979; Rizvi and Rizvi, 1992). In addition, environmental conditions and genetic characteristics are the most effective agents in enhancing synthesis and exudation of allelochemicals (Pramanik et al., 2000). The inhibitory effect of black walnut on neighbouring plants or associated plant species is

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one of the oldest recorded incidences of allelopathy (Kocacaliskan and Teriz, 2001). There are many plants implicated in the allelopathic release of different active substances such as *p*-hydroxy benzoic acid (*Camelina allysum*) that influence flax (Grummer and Beyer, 1960; Barkosky and Einhellig, 2003) and walnut (Prataviera et al., 1983). Hydroxyjuglon is the main active substance of walnut, which when exposed to the air or some oxidizing substance, produces its toxic form, juglon (Segura-aguilar et al., 1992). Other types of allelochemicals are phenolic acids and associated compounds that are common growth inhibitors produced by living plants or released from decaying plants by microbial action or leaching (Fuji et al., 2003). Chala and Rarvindra (1998) report that *Cyperus rotundus* Linn., *Cynodon dactylon* (L) Pers., *Echinochola colonum* (L) Link., *Portulaca oleraceae* L. and *Lagasca mollis* Cav. were the major weeds in all cropping seasons, considerably reducing the yield of vegetables at the Indian Institute of Horticultural Research. Results from the study that used "test plants" such as lettuce and radish are difficult to generalize to other species and are not applicable. Therefore the study of allelopathic effects of some medicinal plants on weeds and other plant species would be useful, not only as a guide for organic culture, but also for rotation programming in medicinal plant production.

The present work aimed to identify the allelopathic effects of hydro-alcoholic extract of sage (*Salvia officinalis*) and St. John's wort (*Hypericum perforatum*) and essential oils of *Eucalyptus globulus* and *Bunium persicum* on *Amaranthus retroflexus*, *Portulaca oleracea*e and radish and lettuce seed germination.

MATERIALS AND METHODS

In this work, we prepared a hydro-alcoholic extract from some medicinal herbs such as *Hypericum perforatum* and *Salvia officinalis*, and prepared an extract of essential oils such as *Eucalyptus globulus* and *Bunium persicum*. The experimental plants were collected and dried in a dark room. After appropriate drying, the plants were kept in an air-tight box until extraction. A 10 g dried sample was homogenized in 100 ml of distilled water by blender and filtered. The filtrate was used in experiments diluting 1/2, 1/5, 1/10 with distilled water or without diluting along with a control. Essential oils of *Eucalyptus globulus* and *Bunium persicum* were extracted by Clevenger apparatus and diluted by Tween 20 and water into a solution of 0.2, 0.5, 0.7 and 1 percent (v/v). The experiment was conducted by using a completely randomized block design (CRBD) with three replicates. Duncan's multiple range test was used for comparing the means of seed germination percent and germination rate. Each petri dish was an experimental plot. The seeds of *Amaranthus retroflexus* and *Portulaca oleraceae* were obtained from the seed bank of Ferdowsi University of Mashhad (FUM). The seeds were surface sterilized with sodium hypo-chloride (1%), and then washed with distilled water three times. At least 30 seeds were placed in a petri dish furnished with sheets of filter paper moistened with hydro-alcoholic solution and plant extract or essential oils as the control and treatments respectively.

RESULTS AND DISCUSSION

Medicinal Plants Extract

The effect of *Hypericum perforatum* and *Salvia officinalis* extract on the seed germination of the weeds is shown in Table 1. The results show that all concentrations of *Hypericum perforatum* extract significantly decreased germination percent of *Amaranthus retroflexus* compared to control. Undiluted *Hypericum* extract completely inhibited *Amaranthus retroflexus* seed germination. Undiluted and 1/5 diluted *Salvia* extracts significantly decreased *Amaranthus retroflexus* seed germination compared to control, but not the other salvia extracts. All treatments significantly decreased the germination rate. The highest germination rate was measured in the control and the lowest one in undiluted *Hypericum* and *Salvia* extracts. *Hypericum perforatum* and *Salvia officinalis* extracts did not have a significant effect on *Portulaca oleracea* seed germination except the undiluted

Hypericum extract that significantly decreased the germination percent and rate of *Portulaca* compared to the control.

Eucalyptus Essential Oil

The effect of *Eucalyptus* essential oils on seed germination is shown in Table 2. Our results show that *Eucalyptus* essential oils significantly decreased germination percent of both weeds compared to the control. Germination of *Amaranthus retroflexus* and *Portulaca oleraceae* was completely inhibited at a concentration of 0.7% (v/v) and higher. There was no significant difference between 0.5 and 0.7% essential oil for *Portulaca oleraceae* germination percent. Therefore *Eucalyptus* essential oils at low concentration are a suitable seed germination suppressant. The germination rate of *Amaranthus retroflexus* and *Portulaca oleraceae* was also affected significantly by the treatments. The highest germination rate was measured in the control (30.6 and 46.7 seeds per day respectively). There was no significant difference between 0.2 and 0.5% *Eucalyptus* essential oils concentrations on the germination rate of both weeds. *Eucalyptus* essential oils have several components such as 1, 8 - cineol that affects the germination processes of the seed (Duke et al., 2002). Romagni et al. (2000) show that 1,8 - cineol inhibits asparagin synthetase activity.

Black Zira Essential Oil

Black Zira *(Bunium persicum)* is a native plant of Iran belonging to the *Apiaceae* family. Its essential oil has been exported to other countries for several purposes. *Bunium persicum* essential oils contain carvon that inhibit the germination of seeds, therefore we conducted a test of its effect on lettuce and radish seeds. Our results confirm that *Bunium persicum* essential oil has a strong inhibitory effect on lettuce and radish germination (Table 3). As shown in Table 3, the germination of *Lactuca sativa* seeds was completely inhibited by 0.2% essential oil of *Bunium persicum,* but for complete inhibition of *Raphanus sativus* seeds, we needed a more concentrated solution (1%). Therefore, lettuce seeds are more sensitive than radish to *Bunium persicum* essential oil. The germination rate of the seeds was also affected by *Bunium persicum* essential oils. Radish seeds in the control treatment showed a higher germination rate (26.7 seeds per day) and there was a significant difference between radish and lettuce seed. Lamoureux and Koning (2004) show that seeds of the *Apiaceae* family contain cuminaldehyde, cuminyl alcohol and fenchone that have a strong inhibitory effect on the germination of lettuce seed.

The cumulative germination percentage (CGP) of *Amaranthus retroflexus* is shown in Figure 1. On the second day after treatment, in the control plot, almost 60% of the amaranth seeds had germinated, but the CGP in seeds treated with 0.2 and 0.5% *Eucalyptus* essential oils were 3.5% and 1.5% respectively. On the tenth day, the CGP in the control treatment was about 85%, but in seeds treated with 0.2 and 0.5% *Eucalyptus* essential oil, it was lower than 10 and 20% respectively. Our results for *Portulaca oleraceae* showed that on the second day after treatment, in the control plot, the CGP was nearly 65%, but it was around 1.5% in treated seeds with 0.2 and 0.5% in *Eucalyptus* essential oils. On the tenth day after treatment, the CGP in the control plot reached 90% but in the *Portulaca oleraceae* seeds treated with essential oil, it was significantly lower (nearly 10%) (Fig. 2). There is no significant difference between 0.2 and 0.5% essential oil treatments on the CGP of *Portulaca* seeds. Therefore *Eucalyptus* essential oils affect the CGP of both weeds.

The effect of *Bunium persicum* essential oil on radish and lettuce seed germination showed similar results. *Bunium persicum* essential oil significantly decreased the CGP of radish and lettuce seeds (Figs. 3 and 4). Our results show that radish seeds are more resistant than lettuce to *Bunium persicum* essential oil because the CGP of radish seeds treated with 0.1% essential oils was higher than lettuce after the second and the tenth day after treatment. The higher the essential oil concentration, the lower the CGP of lettuce and radish seeds. Other researchers have shown the inhibitory effects of medicinal plant essential oils on the germination of radish (Feo et al., 2000), lamsquarters, common

ragweed and Johnson grass (Tworkoski, 2002), wheat (Dudai et al., 2004). Aqueous extracts of medicinal plants inhibited the germination of Picao Preto (*Bidense pilosa*) (Cruze et al., 1999). In conclusion, medicinal and aromatic plants that contain essential oils can be used for weed control in sustainable and organic agriculture as mulch or as a bio-herbicide.

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Tables

Table 1. Effect of different concentrations of *Hypericum perforatum* and *Salvia officinalis* hydroalcoholic extract on seed germination of weeds.

	Amaranthus retroflexus		*Portulaca oleracea*	
	Germination %	Germination rate	Germination %	Germination rate
Undiluted *Hypericum* extract	0 c	0 c	81.3 b	30.7 b
1/5 diluted *Hypericum* extract	60.7 b	16.2 b	96.0 a	48.0 a
1/10 diluted *Hypericum* extract	62.7 b	21.3 b	90.7 ab	45.1 a
1/20 diluted *Hypericum* extract	62.7 b	23.2 b	86.0 ab	42.7 a
Undiluted *Salvia* extract	2.0 c	0 c	91.3 ab	39.0 ab
1/5 diluted *Salvia* extract	61.3 b	15.3 b	89.3ab	43.9 a
1/10 diluted *Salvia* extract	74.7 ab	21.1 b	93.3 ab	46.1 a
1/20 diluted *Salvia* extract	76.0 ab	23.4 b	90.7 ab	44.5 a
Control	84.0 a	35.3 a	91.3 ab	47.1 a
LSD 5%	18.0	9.45	10.9	8.68

Table 2. Effect of different concentrations of *Eucalyptus globulus* essential oils on germination of weeds.

Treatments	Germination%		Germination rate	
	Amaranthus retroflexus	*Portulaca oleracea*	*Amaranthus retroflexus*	*Portulaca oleracea*
1% essential oil	0 e	0 e	0 e	0 d
0.7% essential oil	0 e	0 e	0 e	0 d
0.5% essential oil	8.0 d	9.0 d	1.22 cd	1.18 cd
0.2% essential oil	16.0 c	11.0 d	2.41 c	1.47 c
Control	81.5 b	89.2 a	30.6 a	46.7 a
LSD 5%	4.74		1.18	

Table 3. Effect of different concentrations of *Bunium persicum* essential oils on germination of lettuce and radish.

Treatments	Germination%		Germination rate	
	Lactuca sativa	*Raphanus sativus*	*Lactuca sativa*	*Raphanus sativus*
1% essential oil	0 d	0 e	0 d	0 d
0.5% essential oil	0 d	16.7 c	0 d	1.12 d
0.2% essential oil	0 d	17.5 c	0 d	2.5 d
0.1% essential oil	41.7 b	34.2 b	6.2 c	7.2 c
Control	100 a	100 a	13.8 b	26.7 a
LSD 5%	8.2		3.4	

Figures

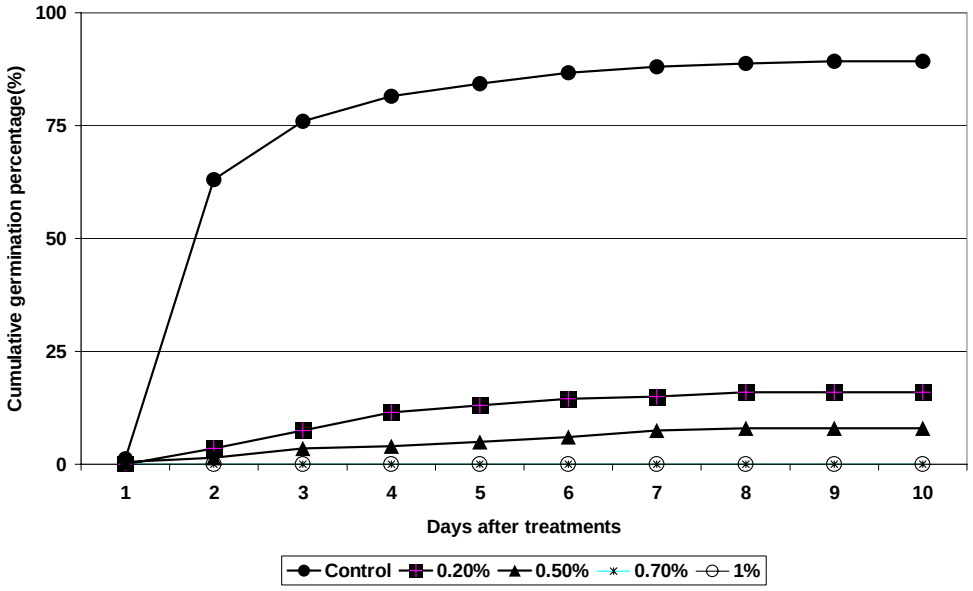

Fig. 1. Effect of different concentrations of *Eucalyptus globulus* essential oil on cumulative germination percentage of *Amaranthus retroflexus.*

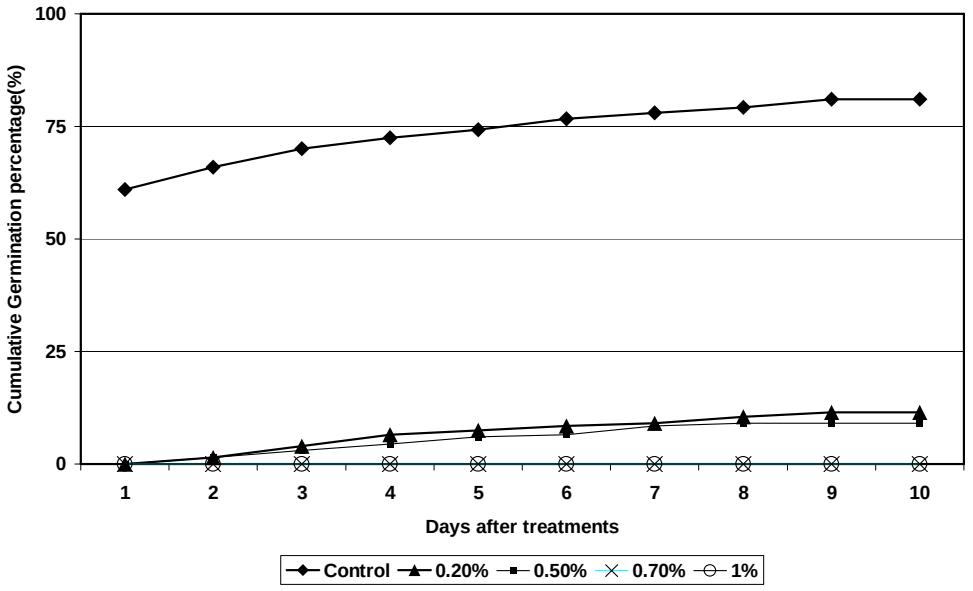

Fig. 2. Effect of different concentrations of *Eucalyptus globulus* essential oil on cumulative germination percentage of *Portulaca oleraceae*.

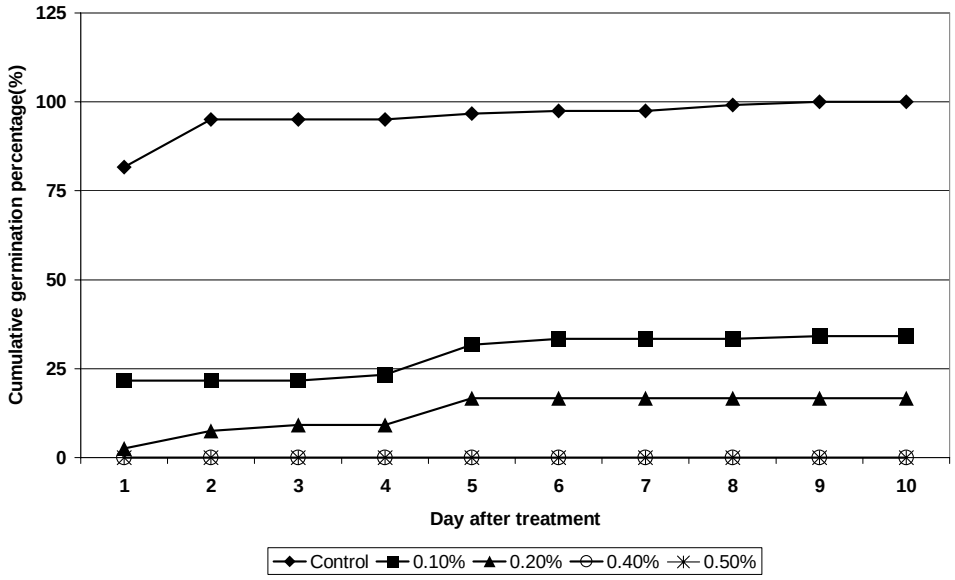

Fig. 3. Effect of different concentrations of *Bunium persicum* essential oil on cumulative germination percentage of radish.

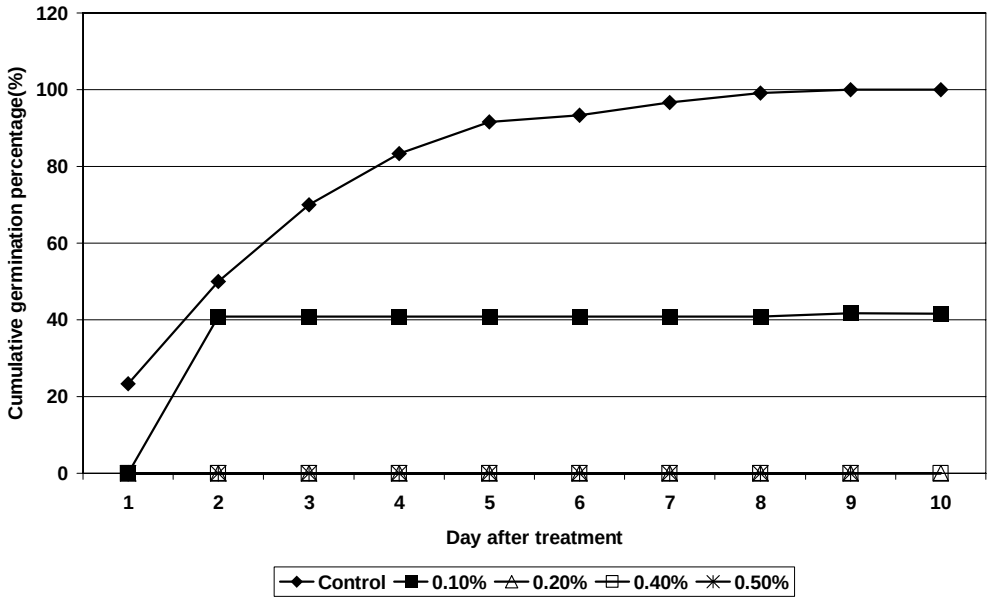

Fig. 4. Effect of different concentrations of *Bunium persicum* essential oil on cumulative germination percentage of lettuce.