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## Allelopathic Effects of *Eucalyptus camaldulensis* on Seed Germination and Initial Growth of four range species

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### ABSTRACT

This research was carried out to study allelopathic effect of aerial and underground part of *Eucalyptus camaldulensis* on germination and early growth seedling of *Vicia villosa*, *Onobrychis sativa*, *Festuca arundinacea* and *Trifolium rigidum*. The extraction was provided from aerial and underground part of *Eucalyptus camaldulensis*. Different concentrations of this species (0, 25, 50, 75 and 100 %) was provided by adding distilled water. Then completely randomized design with five treatments and four replications was used to analyze the data. Results indicated that there were significant differences among all traits (germination percentage, germination speed, root length, shoot length, plant length and seed vigor index) except for germination percentage in *Vicia villosa* ( $P < 0.01$ ). Means Comparison indicated germination percentage and speed and primary growth indices decreased by increasing of extract concentration. The highest germination percentage and speed, length of radical and plumule and seed vigority were belonged to control treatment and the lowest length belonged to 100 % extraction. Germination and early seedling growth of 4 species were reduced significantly by increase of extract concentration so that *Onobrychis sativa* and *Trifolium rigidum* indicated more sensitive to allelopathic effect of *Eucalyptus camaldulensis*.

**Keywords:** Allelopathy, *Eucalyptus camaldulensis*, *Vicia villosa*, *Onobrychis sativa*, *Festuca arundinacea*, *Trifolium rigidum*.

### INTRODUCTION

Allelopathy is defined as direct and indirect effects of allelochemical compound resulted from organism which may have inhibitor or stimulator effects on the same or different organism. In this process, Synthesis of biologically active molecules produced by plant and their residue may convert to other forms and influenced on growth of similar or non similar plants [6]. Allelopathy term was introduced by Molich in 1937. He defined it as reciprocal effects of biochemical compounds among all plants and microorganism. More studies on allelopathy were conducted on germination percentage and speed and flowed by early seedling growth [13]. Plants produce many chemical compounds in their growth period. These compounds release in environment in form of gases and or by leaching from aerial body, leaking from root and or decomposing plant resides. Frequently allelopathy caused decrease in plant growth more than what caused by competition in plants on sunlight, water and nutrition [7]. Allelopathic compounds restrict plant growth through negative interactions with important physiological processes such as changes in cell wall structure, prevention of cell division and activity of some enzymes. These compounds can also affect the equilibrium of plant hormones, pollen tube germination, absorption of nutrient elements, displacement of stomata,

photosynthesis, respiration, protein synthesis, pigment, and changes in DNA and RNA structures [8]. Allelopathic effect of Umbelliferae family on lotus was verified by [12, 14] investigated the allelopathic effect of *Artemisia annua* on redroot pigweed (*Amarantus retroflexus*), common lambsquarters (*chenopodium album*), soybean (*Glycine max*) and corn (*Zea mays*). They reported that this species had inhibitor effect on studied plant and it caused a decrease on weight of aerial parts. The effects of four lettuce (*Lactuca sativa*) cultivar leaf extracts were examined on brandyard grass. It was reported that leaf residues of lettuce inhibited root and shoot fresh weights by 88% and 79%, respectively [25]. Inhibitory effects of *Justicia anselliana* (Nees) T. Anderson on *Vigna unguiculata* (L.) was tested by [5]. They found that all isolated compounds obtained from *Justicia anselliana* showed an inhibitory effect on the three parameters measured on *Vigna unguiculata* germination (rate of germination, shoot length, and fresh weight). [9] reported that aqueous extracts of *Thymus kotschyanus* had a considerable inhibitory effect on germination of *Bromus tomentellus* and *Trifolium repens*. [1, 4] also studied the allelopathic effect of *Thymus kotschyanus* on *Achillea millefolium* and *Sanguisorba minor* respectively. Euphorbia family also is one of the other allelopathic plants. [24] reported that *Euphorbia geniculata*, *E. hirta* and *E. microphylla* exhibited inhabitation in germination percentage in initial stage of *triticum aestivum*.

*Eucalyptus camaldulensis* is one of the most important plants used to prevent soil erosion and to recover the plant cover in studied area. This plant also used in farmland as windbreak and medical plant. In studied area which consisted of 6000 hec, many plants (range species, cultivatable and medical plant) were cultivated based on different goals. Understanding allelopathic effect of plants on each other helps managers correctly to plan their vegetative projects for restoring and rehabilitating of destructive environments. The objective of this research was to investigate the allelopathic effect of *Eucalyptus camaldulensis* on germination and early seedling growth of four range species.

### MATERIALS AND METHODS

Aerial and underground parts of *Eucalyptus camaldulensis* were collected from Chah Nime, Zabol, Iran. After air drying at room temperature, 5 g of powder were picked and mixed in 100 ml water and placed on a shaker for 24 h and then centrifuged at 3000g for 15 min. The obtained mixture was filtered using Whatman 1 filter paper. Concentrations of 25, 50, 75 and 100% were prepared using the centrifuged solution and effect of these concentrations were tested on *Vicia villosa*, *Onobrychis sativa*, *Festuca arundinacea*, *Trifolium rigidom*. Distilled water was used as control treatment. Plant seeds were first disinfected using a 10% solution of sodium hypochlorite then washed with distilled water several times. Plastic Petri dishes were also sterilized for 2 h in the oven at 150°C. Then 25 disinfected seeds were placed into Petri dishes with a 9 cm filter paper (Whatman 1) and sprayed 5 ml of each extracts.

The germination test was performed using a completely randomized design with four treatments with four replications (25 seeds per replication). The experiment was conducted at seed ecophysiology laboratory, department of range and watershed management, Zabol university in July 2011. Germinated seeds of more than 2mm length were counted each day over 10 days and the germination percentage, germination speed, root length, shoot length, plant length and seed vigour index were measured. Germination percentage [11], germination speed [10] and seed vigour index [20] were calculated based on the following equations:

$$(1) \text{ Germination percentage: } GP = \frac{\sum G}{N} \times 100$$

where Gp is germination percentage, G is the number of germinated seeds and N is the number of seeds.

$$(2) \text{ Germination speed: } GR = \sum_{i=1}^n \frac{S_i}{D_i}$$

where  $S_i$  is the number of germinated seed at each counting,  $D_i$  is the number of day until n counting and n is the number of counting.

$$(3) \text{ Vigour index} = \text{Total germination percentage} - \text{Plant length.}$$

$$(4) \text{ Plant length} = \text{Root length} + \text{Shoot length.}$$

The collected data were analyzed using MSTAT-C and mean comparison was performed using Duncan's Multiple Range Test. Figures were drawn by excel.

## RESULTS

Results of analysis of variance indicated that extract of *Eucalyptus camaldulensis* had significant effect on germination and early seedling growth of *Vicia villosa*, *Onobrychis sativa*, *Festuca arundinacea*, *Trifolium rigidom* (Tables 1, 2, 3 and 4).

**Table 1. Variance analysis of studied traits of *Vicia villosa***

Properties	ss	df	ms	F
Germination percentage	570	4	142.5	0.95 <sup>ns</sup>
Germination speed	12.1	4	3.04	6.8 <sup>**</sup>
Root length	98.09	4	24.5	88 <sup>**</sup>
Shoot length	18.6	4	4.6	16.1 <sup>**</sup>
Plant length	201.3	4	50.3	58.7 <sup>**</sup>
Seed vigourity	1760994.8	4	440248.7	22.4 <sup>**</sup>

\*\* : significant differences between treatments at 1% level; ns: non-significant difference between treatments.

**Table 2. Variance analysis of studied traits of *Onobrychis sativa***

Properties	ss	df	ms	F
Germination percentage	2400	4	600	5.2 <sup>**</sup>
Germination speed	11.9	4	2.9	12.6 <sup>**</sup>
Root length	3.1	4	0.7	16.2 <sup>**</sup>
Shoot length	1.6	4	0.4	22.4 <sup>**</sup>
Plant length	9.1	4	2.2	34.3 <sup>**</sup>
Seed vigourity	83518.2	4	20879.5	14.2 <sup>**</sup>

\*\* : significant differences between treatments at 1% level.

**Table 3. Variance analysis of studied traits of *Festuca arundinacea***

Properties	ss	df	ms	F
Germination percentage	6580	4	1645	51.9 <sup>**</sup>
Germination speed	2.01	4	0.5	59.3 <sup>**</sup>
Root length	31.5	4	7.8	422.03 <sup>**</sup>
Shoot length	52.08	4	13.02	36.9 <sup>**</sup>
Plant length	164.3	4	41.08	98.9 <sup>**</sup>
Seed vigourity	693422.5	4	173355.6	122.9 <sup>**</sup>

\*\* : significant differences between treatments at 1% level.

**Table 4. Variance analysis of studied traits of *Trifolium rigidom***

Properties	ss	df	ms	F
Germination percentage	10480	4	2620	18.7 <sup>**</sup>
Germination speed	5.9	4	1.4	48.6 <sup>**</sup>
Root length	26.6	4	6.6	14.8 <sup>**</sup>
Shoot length	13.2	4	3.3	19.4 <sup>**</sup>
Plant length	76.9	4	19.2	16.5 <sup>**</sup>
Seed vigourity	371669.2	4	92917.3	24.3 <sup>**</sup>

\*\* : significant differences between treatments at 1% level.

### Germination percentage

Mean comparison indicated that germination percentage in all species decreased by increasing the concentration. There was significant differences among germination percentage at all species except for *Vicia villosa* ( $P > 0.01$ ). The highest germination percentage was belonged to control treatment and the lowest one belonged to 100 % extraction (fig. 1).

### Germination speed

Effect of different extract concentrations of *Eucalyptus camaldulensis* was significance on germination speed so that germination speeds of *Vicia villosa*, *Onobrychis sativa*, *Festuca arundinacea*, *Trifolium rigidom* decreased by increasing of concentration. The highest and lowest germination speed was belonged to control and 100 % extraction respectively (fig. 2).

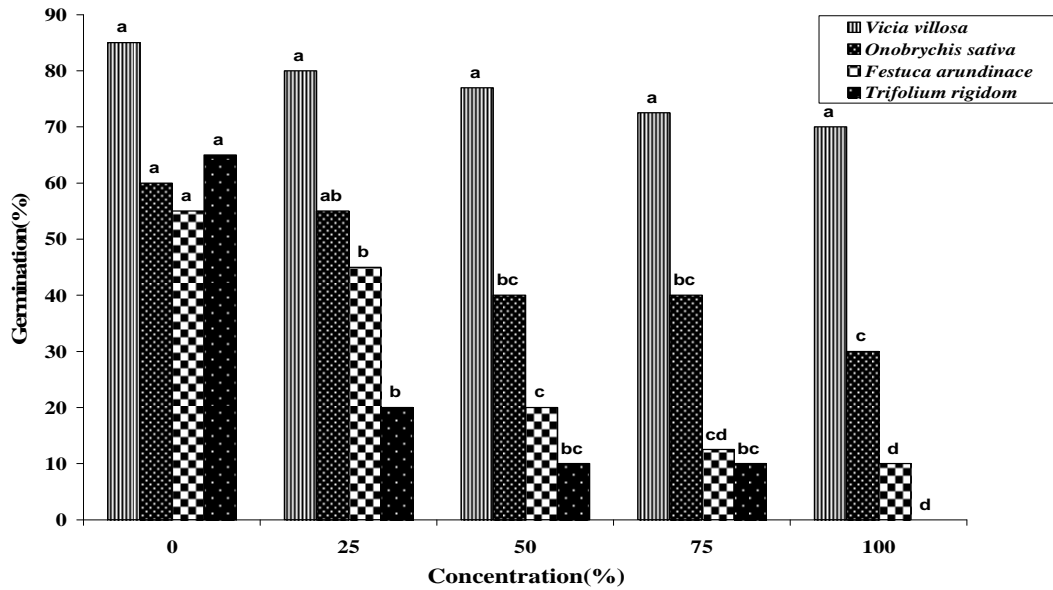


Figure 1. comparison the allelopathic effect of *Eucalyptus camaldulensis* on germination percentage of studied species.

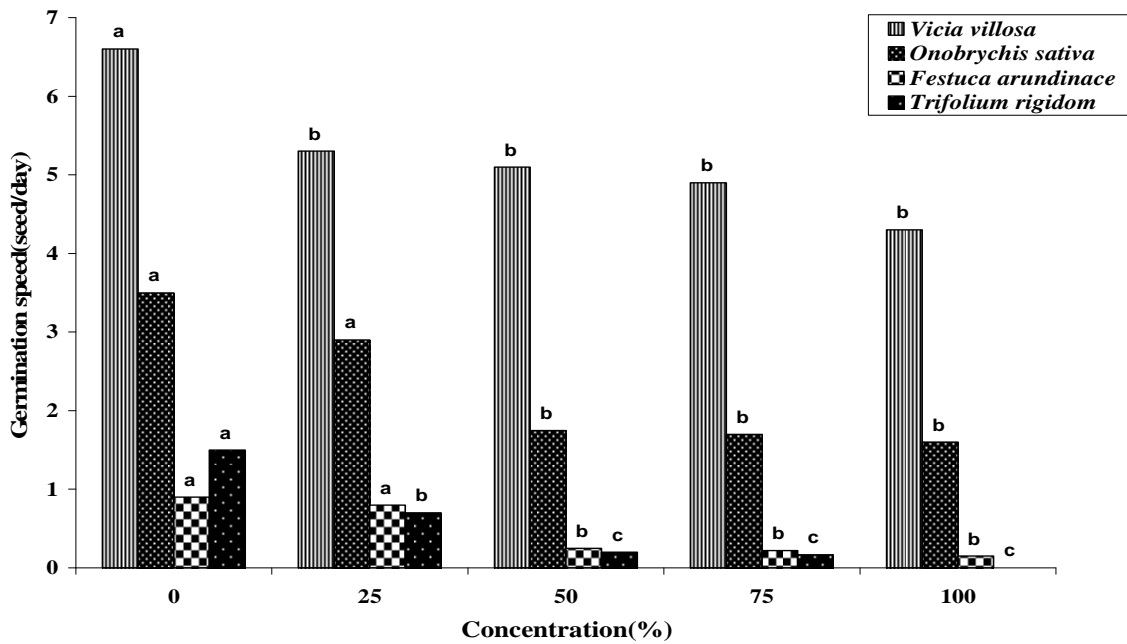


Figure 2. Comparison the allelopathic effect of *Eucalyptus camaldulensis* on germination speed of studied species.

**Root length, shoot length and plant length**

Figures 3, 4 and 5 indicate that there is significant differences among length of root, shoot and seedling respectively ( $p < 0.01$ ). Results indicate that length of root, shoot and plant length decrease by increasing of extract concentration (figs. 3, 4 and 5).

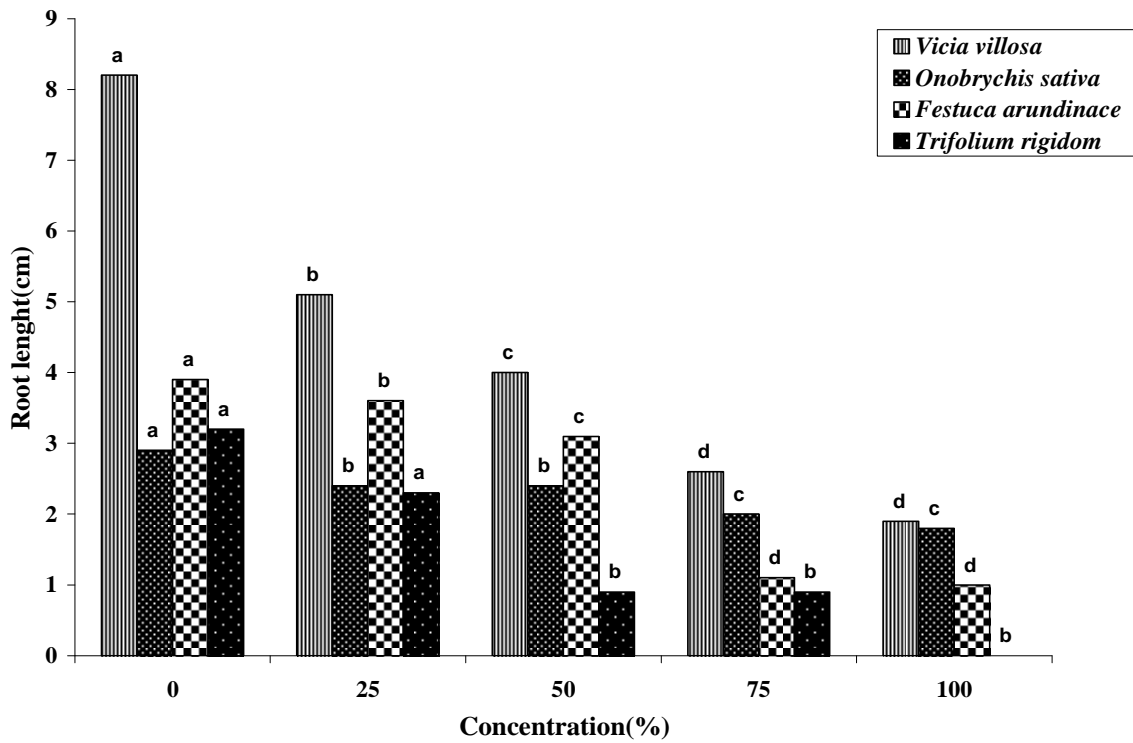


Figure 3. Comparison the allelopathic effect of *Eucalyptus camaldulensis* on root length of studied species.

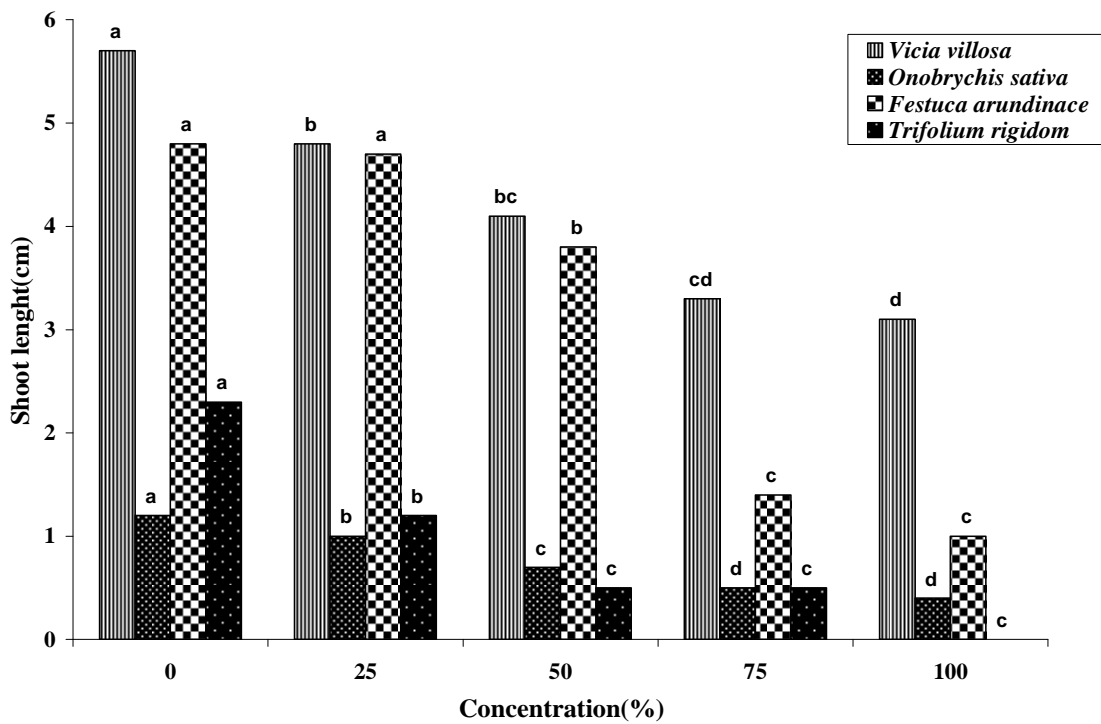


Figure 4. Comparison the allelopathic effect of *Eucalyptus camaldulensis* on shoot length of studied species.

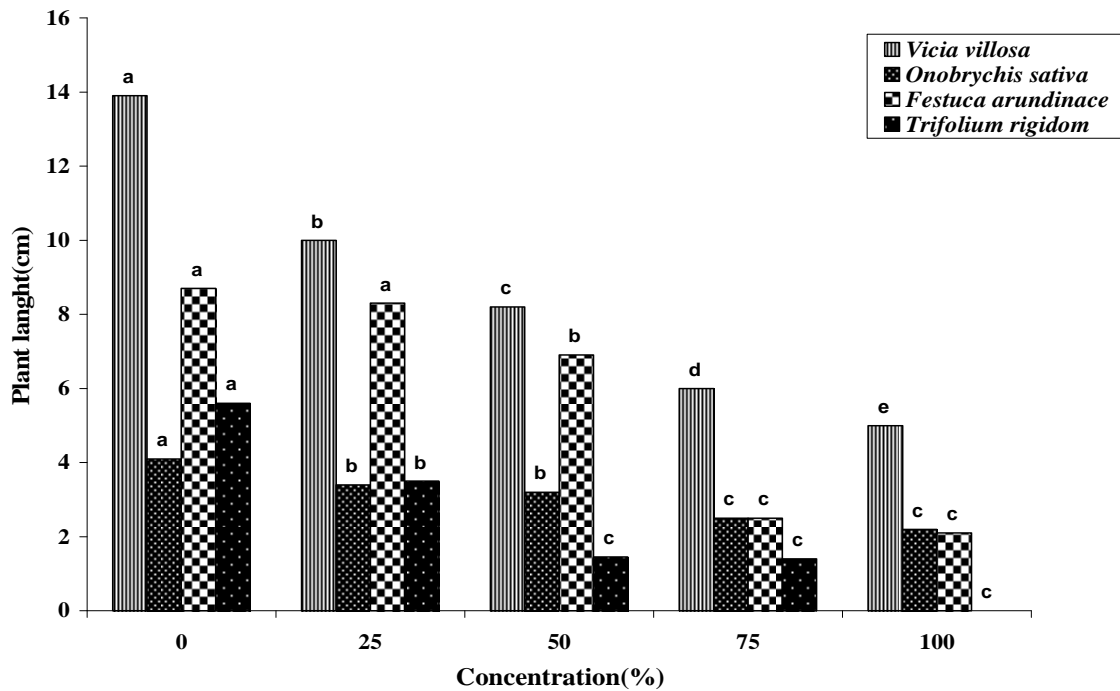


Figure 5. Comparison the allelopathic effect of *Eucalyptus camaldulensis* on plant length of studied species.

**Seed vigor index**

Effect of different extract concentrations of *Eucalyptus camaldulensis* were significance on Seed vigour index so that seed vigourity of *Vicia villosa*, *Onobrychis sativa*, *Festuca arundinacea*, *Trifolium rigidom* decreased by increasing of concentration. The highest and lowest seed vigourity was belonged to control and 100 % extraction respectively (figure 6).

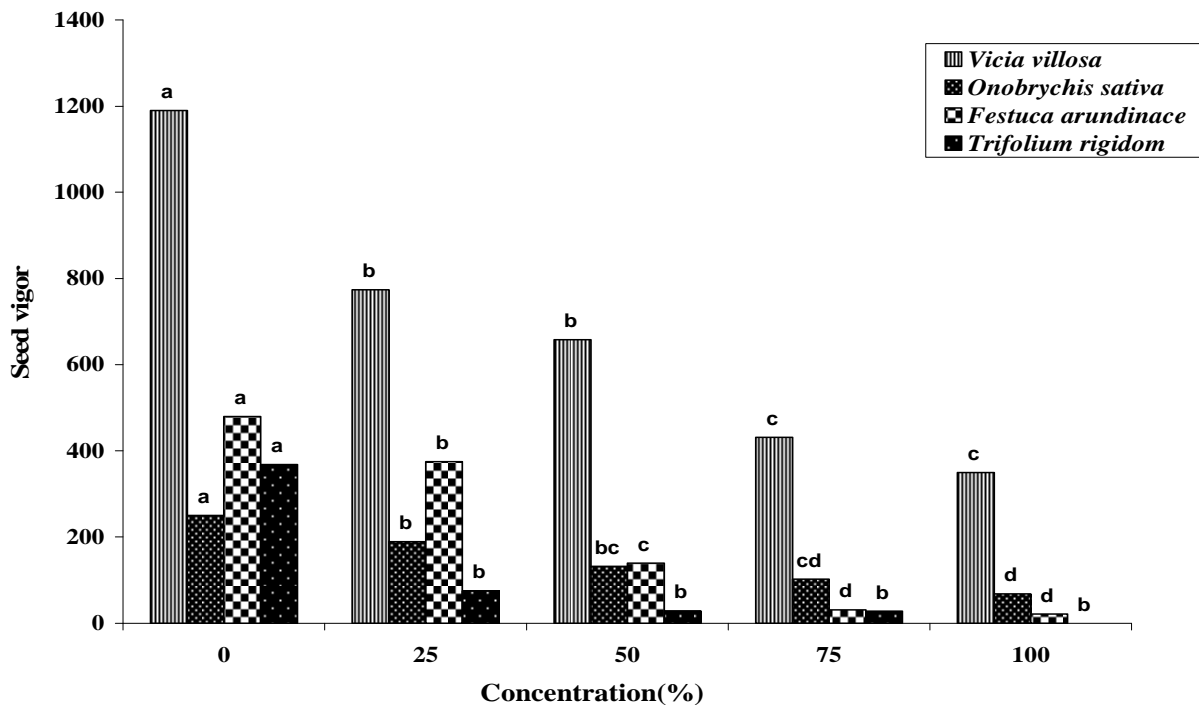


Figure 6. Comparison the allelopathic effect of *Eucalyptus camaldulensis* on seed vigor index of studied species.

**DISCUSSION AND CONCLUSION**

Results indicated that aerial and underground extract of *Eucalyptus camaldulensis* could affect on germination and early seedling growth of *Vicia villosa*, *Onobrychis sativa*, *Festuca arundinacea* and *Trifolium rigidom*. *Trifolium*

*rigidom* and *Onobrychis sativa* showed more sensitivity to allelopathic effects of *Eucalyptus camaldulensis* than those of other species. Increase in extract concentration had significant effect on decrease of studied parameters in four species which coincided with the research of [9, 15, 17, 22, 23, 26]. Decrease of germination can be caused by inhibitive effect of allelochemical compounds on hormones especially gibberellin. Stopping of germination during germination period may also be because of changing in enzyme activities which restricted the conversion of nutritive compounds [3]. Delay or stimulate in using nutritive matter can cause lack of Production of Respirable Vesicles and finally result in lack of ATP in seeds exposed to allelochemical compounds. [21] stated that metabolic energy restricted by disorder in respiration rate and consequently decreased the early growth of seedling. Radicle and plumule are the first organs emerged from the seed and exposed to allelopathic matter since it can be predictable that their growth decrease when they expose to allelopathic compounds. Increase in extract concentration decreased the length of plumule and put a stop on it at the highest level of extract (100 %). Decrease in plumule length can cause by inhibiting in cell division and elongation and or decreasing in hormones such as acetic acid and gibberellin. Consequently these may cause short and weakness seedlings and then decrease the establishment of them. [2] stated that decrease in plumule and radicle length might be because of decreasing in cell division. Allelochemical decreased amount of fusion oxine in roots [23]. These compounds decrease the growth by hindering nutritive absorption and or interfering in respiration [7]. Chemical matter with allelopathic impact influences the germination and early seedling growth by two ways. First, they hinder the cell division and second they inhibit the elongation of cells [18]. [15] reported that many of allelopathic compound could decrease stimulate effect of hormones such as gibberellin and acetic acid. Furthermore allelopathic compound could disorder in vital activities of plants by other mechanisms such as restriction of nutritive absorption [11], disorder in respiration, oxidative phosphorylation and photosynthesis [18].

According to this research *Eucalyptus camaldulensis* have allelopathic effect on studied species and it can damage germination and early seedling growth of planted seeds. Because of important of primary growth stage on establishment of plant, it suggested that the mentioned plants don't cultivate with *Eucalyptus camaldulensis*. *Eucalyptus camaldulensis* have medic values and cannot be eliminated from the studied area hence we must pursuit the proper way to eliminate the allelopathic effect of this species on the others. This experiment was conducted in laboratory condition therefore it suggests that more research could be carried out in greenhouse condition because in natural condition the results may change as a result of differences in growth conditions. It also suggested that more investigation about the allelopathic effect of this species should be carried out on the other species.

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