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Allelopathic Effects of Introduced *Eucalyptus Gomphocephala* Extracts on Germination and Seedling Growth of Native Ceratonia siliqua in Libya

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Abstract

The present study aimed to investigate the allelopathic effects of leaf aqueous extracts of introduced *Eucalyptus gomphocephala* L. (donor species) were collected during different growing seasons at different concentrations (0, 25, 50, 75, and 100% v/v) on germination percentage, average germination time, plumule and radical length, dry weight of radical and plumule and seedling vigor index (SVI) of *native Ceratonia siliqua* L. (recipient species) seedlings under laboratory conditions. Results suggested that the germination percentage and all growth parameters of *C. siliqua* were significantly reduced gradually with the increase of aqueous extract concentration levels and the reduction was varied and could be extract concentration and growing season dependent. The highest phytotoxic effect on germination and seedling growth was exhibited by *E. gomphocephala* aqueous extract at 100% concentration, followed by 50% concentration. Generally, it is concluded that the allelopathic effect of introduced *E. gomphoracephala* trees on native *Ceratonia siliqua* trees has been weak or no effectiveness in some cases under laboratory conditions.

Keywords: *Eucalyptus gomphocephala;* Introduced species; Libya; Allelopathy; *Ceratonia siliqua*

Introduction

An introduced species (also known as an exotic species) is an organism that is not native to the place or area where it is considered introduced and instead has been accidentally or deliberately transported to the new location by human activity. Invasive species are introduced species that are very successful in their new habitat, reach large population sizes, and have negative impacts on native species including reduced germination, growth, survival, and reproduction (*1*). When a non-native species is introduced into a new environment it is freed from the natural predators, parasites, or competitors from its native habitat. This gives an advantage to non-native species competing with the native species that evolved in the ecosystem (*2*). Allelopathy is a biological phenomenon that means that plants can release some biochemical substances into the surrounding environment, which can be inhibitory impacts on other plants directly or indirectly (*3*). Allelopathic inhibition is complex and can involve the interaction of different classes of chemicals like phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids, with mixtures of different compounds sometimes having a greater allelopathic effect than individual compounds alone (*4*). Furthermore, physiological and environmental stresses, pests and diseases, solar radiation, herbicides, and less than an optimal nutrient, moisture, and temperature levels can also affect or modify allelopathic activities. Different plant parts, including flowers, leaves, leaf litter, and leaf mulch, stems, bark, roots, soil and soil leachates, and their derived compounds, can have allelopathy activity that varies over a growing season (*5*). Eucalyptus *gomphocephala* Family: *Myrtaceae* is an evergreen tree native to the Mediterranean climate of southwestern Australia; it can grow 10 - 45 meters tall (*6*). Eucalyptus gomphocephala, is an introduced (exotic) tree species that were planted in the Jabal Akhdar region in the 1930s (*6*).

E. gomphocephala is one of the species most widely found in the west of Libya, where it has been successfully planted (*7*). Various *Eucalyptus* species have been found to change the composition of native communities and has been associated with reduced plant and insect diversity (*8*). Researchers found that allele-chemicals released by introduced eucalyptus trees can bring inhibitory action to seed germination and seedling growth of the plants around. Allelopathy of Eucalyptus makes the understory shrubs. And herbs become scarce and makes the decline of biodiversity in forests and simple community structure, which will directly cause serious water losses and soil erosion (*9*). Therefore, this study was conducted primarily to determine the Allelopathic effects of leaf aqueous extract of introduced *Eucalyptus gomphocephala* L. (donor species) were collected during different growing seasons on germination and seedling development of native *Ceratonia siliqua* L. (recipient species) in laboratory Petri-dish experiments.

Materials and Methods

Plant materials

The Fresh leaves samples were collected from *Eucalyptus gomphocephala* L. trees that growing in Wadi-Alkufe (East of Libya) during the 2016-2017 and within four seasons. The collected fresh leaves samples were washed and air-dried and then powdered using a grinder and stored at room temperature. The seeds of *Ceratonia siliqua* L. were collected from the same area in Wadi Alkufe in September 2016.

Preparation of plant extracts

The stock aqueous extract was obtained by soaking 100 g of the air-dried leaves in one liter of distilled water at room temperature (20 ±2ºC) for 72 hours. The mixture was filtered and kept in the dark at 5 ºC until use. Different concentrations (25, 50, 75, and 100% v/v) were prepared from the stock solution in addition to the control (distilled water).
Germination bioassay:

Ten seeds of the *C. siliqua* were will be mechanically scarified by knife and arranged in 9-cm diameter Petri-dishes on two discs of Whatman No.1 filter paper under normal laboratory conditions with day temperature ranging from 20-23ºC and night temperature from 14-16ºC. Five milliliters of each level of the donor species aqueous extract concentrations (25, 50, 75, and 100% v/v) were added to three replicates. Before sowing, the seeds were surface sterilized by soaking for two minutes in 10% sodium hypochlorite, then, thoroughly washed with tap water several times followed by distilled water. Treatments were arranged in a completely randomized design with three replications. Seeds were considered germinated when radicle length was 2 mm and the number of germinated seeds was recorded daily, while Germination percentages (GP %), and the average germination time (AGT) was calculated based on the following equations:

Germination Percentage: $GP = \frac{\sum G}{N} X100$

Where GP is germination percentage, G is the number of germinated seeds and N is the number of seeds (*10*). Average germination time (AGT) = Number of germinated seeds x days / ∑ germinated seed (*11*).

The germinated seeds of *C. siliqua* were kept to develop into seedlings for another two weeks. Aqueous extracts were added whenever they were needed. The radicle, plumule growth, the dry weight of radical, plumule and seedling vigor index (SVI) were recorded after 21 days at the end of the experiment. (SVI) = [seedling length (cm) × germination percentage] (*12*).

Statistical analysis

All Data were analyzed using one-way analysis of variance (ANOVA) to compare means of treatments. Also, LSD was used to determine the main differences between the treatment means. All statistical analysis was done using SPSS (version 19.0). Significant differences between treatment means were compared at a 5% probability level.

Results and Discussion

The allelopathic effects of different concentrations of *Eucalyptus gomphocephala* leaf *aqueous* extract were collected during four seasons on the seed germination percentage (GP) of *Ceratonia siliqua* are represented in Table1. Commonly, GP was decreased significantly with the increase of seasonal treatment concentrations level. obviously, the reduction was noticeable in seedlings were treated with leaf aqueous extract that were collected in spring and summer seasons, while other seasons (winter and autumn) have no significant differences in (GP) under different concentrations level (Table1). The data in Table 2 indicated that the average germination time (AGT) was increased gradually with the increase of seasonal treatment concentrations level compared to control. The highest significant inhibitory effect was obvious with summer and winter more than spring treatments concentrations level (5.68, 5.61, and 5.26 seed/day) at 100% treatments concentrations level respectively, while there was no significant inhibitory effect recorded in autumn season (Table 2). Clearly, SVI was decreased significantly with the increase of seasonal treatments concentrations levels, the reduction in (SVI) was (441.46, 354.55, 255.68, and 198.04) in summer, spring, winter, and autumn under 100 % concentrations level respectively (Table 3). Noticeably, all treatment concentration levels had a highly significant reduction in *plumule* and radical length. Actually, at the control level, the *plumule* length was 8.01, 10.28, 10.99, and 12.05 cm, were reduced gradually to 3.58, 4.74, 5.82, and 7.93 cm at 100% concentration level of leaf *aqueous* extract from the donor species during four seasons respectively (Fig.1). Obviously compared to control, all treatment concentration levels had a highly significant reduction in radical length. (Fig.2). The results also demonstrated that the *plumule* dry weight of *C. siliqua* seedlings was high significant effects by the increase in treatment concentration levels during autumn and summer, however, the difference was not significantly relevant to the winter and spring season (Fig. 3). Also, the data indicate that the radical dry weight of *C. siliqua* seedlings was high significantly affected by the increase in treatment concentration level during winter, spring, and summer, on the contrary, the difference was not significantly related to the autumn season (Fig. 4).

Table 1: Effect of different concentrations levels of *E. gomphocephala* leaf aqueous extract on germination percentages of *C. siliqua* during four seasons. Data are mean of three replicates (±SE).

Seasons Conc. %	Autumn	Winter	Spring	Summer	Total G%
00	54.7 ± 9.43	65.24 ± 9.73	* 69.52 ± 9.87 a	* 80.9 ±7.23 a	*** 67.62 ± 4.60 a
25	46.6 ± 8.57	58.10 ± 9.85	66.19 ± 9.72 ab	69.5 ± 9.47 ab	60.12 ± 4.72 a
50	46.6 ± 8.46	54.29 ± 9.92	65.71 ± 9.58 ac	63.8 ± 9.89 ac	57.62 ± 4.73 ab
75	45.2 ± 8.50	47.14 ± 9.36	43.81 ± 7.25 bcd	50.4 ± 9.12 bc	46.67 ± 4.23 bc
100	44.7 ± 8.33	43.33 ± 8.60	37.14 ± 5.78 d	42.3 ± 8.31 c	41.90 ± 3.86 c
L.S.D	F = 0.222 P < 0.926	F = 0.839 P < 0.503	F = 2.990 P < 0.01	F = 2.982 P < 0.01	F=5.490 P<0.001

Different letters within each column indicate a significant difference at P < 0.05 level. *: P < 0.05 ***: P < 0.001

Table 2: Effect of different concentrations levels of *E. gomphocephala* leaf aqueous extract on average germination time (seed/day) of *C. siliqua* during four seasons. Data are mean of three replicates (±SE).

Seasons Conc. %	Autumn	Winter	Spring	Summer	Total (AGT)
0.0	5.47 ± 0.02	*** 5.18 ± 0.03 a	* 5.05 ± 0.01 a	*** 4.62 ± 0.14 a	*** 5.08 ±0.097a
25	5.57 ± 0.15	5.38 ± 0.04 b	5.15 ± 0.05 ab	5.04 ± 0.04 b	5.28 ±0.072 b
50	5.56 ± 0.09	5.5 ± 0.05 bc	5.15 ± 0.03 ab	5.24 ± 0.02 b	5.36 ± 0.057b
75	5.57 ± 0.15	5.65 ± 0.09 c	5.29 ± 0.08 b	5.52 ± 0.09 c	5.51± 0.062c
100	5.59 ± 0.10	5.61 ± 0.04 c	5.26 ± 0.03 b	5.68 ± 0.07 c	5.53± 0.056c
L.S.D	F = 0.172 P < 0.948	F = 11.753 P < 0.001	F = 4.367 P < 0.01	F = 27.283 P < 0.001	F = 22.387 P < 0.001

Different letters within each column indicate a significant difference at P < 0.05 level. * : P < 0.05 *** : P < 0.001

Table 3: Effect of different concentrations levels of *E. gomphocephala* leaf aqueous extract on seedling vigor index of *C.siliqua* during four seasons. Data are mean of three replicates (±SE).

Seasons Conc. %	Autumn	Winter	Spring	Summer	Total seedling vigor index
0.0	*** 685± 28.80a	*** 976± 136.56a	*** 1026± 13.56a	*** 1748± 43.63a	*** 1109± 122.14a
25	572± 61.37a	635± 20.72b	694± 10.42b	1045± 33.54b	737± 57.47b
50	315± 9.50b	607± 26.97b	555± 34.65c	702± 64.21c	545± 46.11c
75	247± 44.70b	371± 40.88c	339± 41.92d	501± 21.37d	364± 31.89d
100	198± 18.83b	255± 33.24c	354± 64.42d	441± 54.61d	312± 34.35d
L.S.D	F = 32.34 P < 0.001	F = 17.11 P < 0.001	F = 54.17 P < 0.001	F = 135.6 P < 0.001	F=175.6 P<0.001

Different letters within each column indicate a significant difference at P < 0.05 level. *** : P < 0.001.

Allelopathy plays an important role in plant interaction in some plant species. According to Rice (*13*), several phytotoxic substances causing germination and/or growth inhibitions have been isolated from plant tissues and soils. Allelopathy has been suggested as a strategy used by many invasive plants, to become dominant in their invaded plant communities (*14*). Seed germination is considered the most critical stage of plant development and, thus, the use of germination indices enables the testing of allele-chemical effects on the physiological germination process (*15*). In the present study, the aqueous extract of the donor species *E. gomphocephala* suppressed seed germination of the recipient species *C. siliqua* under different concentrations. The degree of inhibition was enhanced by increasing the concentration. Moreover, the germination percentage of the recipient species demonstrated a gradual decrease with applying higher concentrations of the donor species regardless of the season. Noticeably, this variant response to the allelopathic substance could be related to the species-specific growth regulatory effect of allelochemicals and concentration-dependent (*16*). Delay in seed germination of many species has important biological implications, accordingly, the establishment of seedlings in natural conditions and their chances of competing for resources with neighboring species will be influenced (*17*).

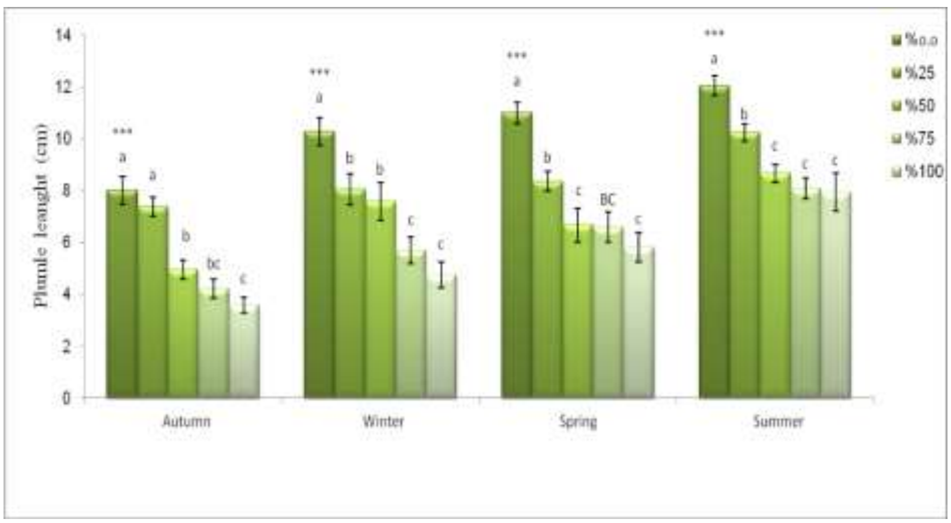

Figure 1: Effect of different concentrations levels of *E. gomphocephala* leaf aqueous extract on plumule length of *C. siliqua* seedlings during four seasons. Different letters within each column indicate a significant difference at P < 0.05 level. ***: P < 0.001.

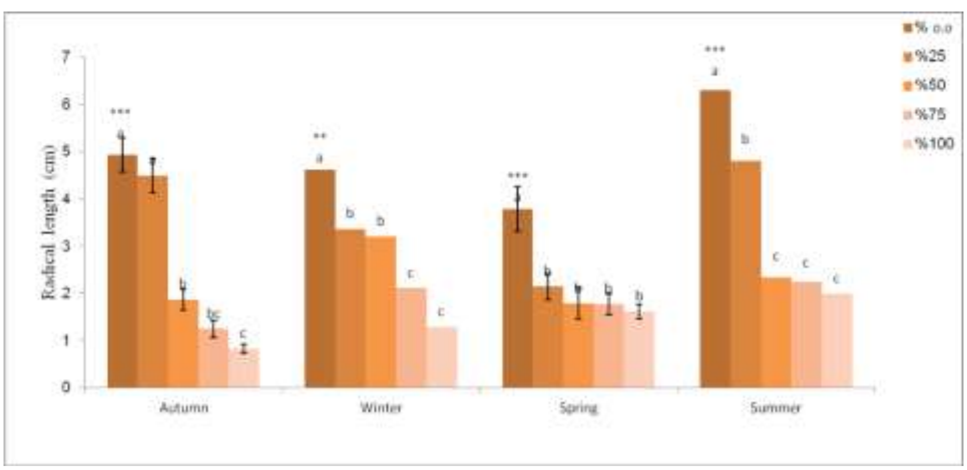

Figure 2: Effect of different concentrations levels of *E. gomphocephala* leaf aqueous extract on radical length of *C. siliqua* seedlings during four seasons. Different letters within each column indicate a significant difference at P < 0.05 level. **: P < 0.01 ***: P < 0.001.

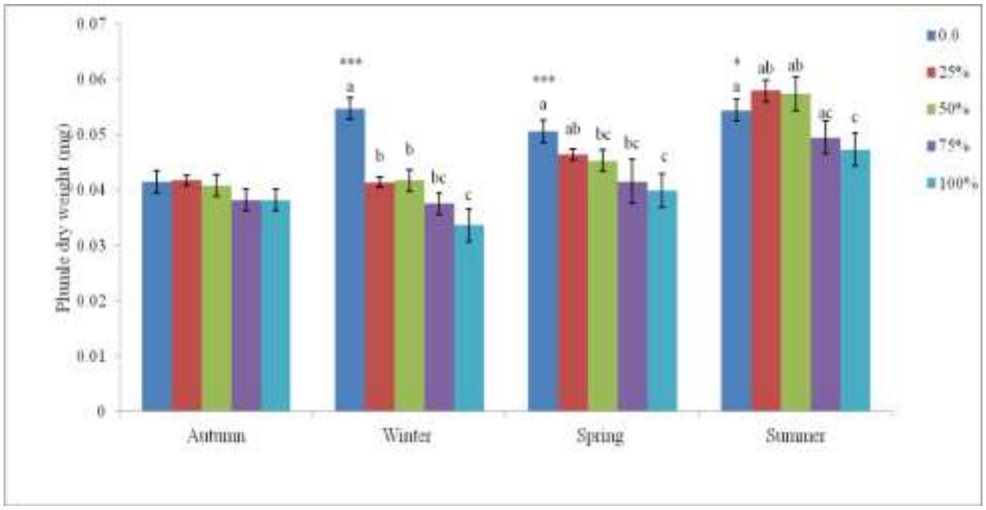

Figure 3: Effect of different concentrations levels of *E. gomphocephala* leaf aqueous extract on plumule dry weight of *C. siliqua* seedlings during four seasons. Different letters within each column indicate a significant difference at P < 0.05 level. *: P < 0.05 ***: P < 0.001.

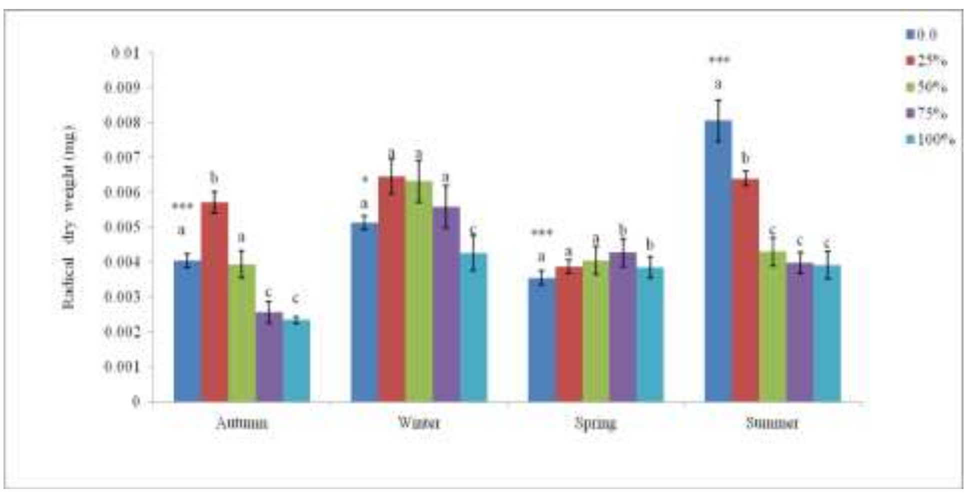

Figure 4: Effect of different concentrations levels of *E. gomphocephala* leaf aqueous extract on the radical dry weight of *C. siliqua* seedlings during four seasons. Different letters within each column indicate a significant difference at P < 0.05 level. *: P < 0.05 ***: P < 0.001.

Also, the results of the current study suggested that aqueous extracts of the donor species exhibited a phytotoxic effect on *plumule* and radicle length of the recipient species under all aqueous extracts concentration levels. These results are in agreement with the declaration that water extracts of allelopathic plants generally have more pronounced effects on a radicle, rather than *plumule* growth. This may be attributable to the fact that radicles have direct contact with soil and can absorb many allele chemicals (*18*). In addition, Salam and Kato-Noguchi (*19*) reported that the higher root growth inhibition is mainly because the roots are the first organ to absorb allele-chemicals from the environment, and the permeability of allele-chemicals into root tissue is higher than the shoot tissue. Furthermore, allele chemicals could be inhibited the elongation, expansion, and division of cells which is a prerequisite for seedling growth (*20*). Several studies have shown that compounds of plant origin, such as allele-chemicals, affect the mitotic activity of growing roots (*21*). Also, under the present study, the aqueous extract of all donor species organs reduced the P-value dry weight (PDW), radical dry weight (RDW), and seedling vigor index (SVI) of the recipient species at different concentration levels. Again, reduction increases as the aqueous extract concentration level of the donor plant increases; however the reduction degree was varied and could be extract concentration and season dependent. This reduction may be attributed to the presence of allele chemicals in the aqueous extracts. These results are in accordance with other studies which reported that *phytotoxicity* may vary among plant parts (*22*). Previous investigations reported that *aqueous* extract of some plant species contains *phenolics* or other toxic substances. They inhibit the germination process by their interference with gibberellins and indole acetic acid, which regulates enzymatic activity during seed germination and embryo growth (*23*). These findings were congruent with other studies on allelopathic effects of *E. globulus* on different crop plants (*24*). As mentioned earlier, this inhibitory effect may be related to the release of *phytotoxic phenolics* from leaf extract, and the toxicity might be also due to a synergistic effect rather than a single one (*11*). The *phytotoxic* effect is probably due to the presence of secondary metabolites such as *alkaloids*, *steroids*, f*lavonoids*, *tannins*, *phenols*, *saponin*, *terpenoids*, volatile oils, and fatty acids in an a*queous* extract from aerial parts of *E. gomphocephala* (*24*). The *phytotoxins* present in plants' *aqueous* extracts, affect the various physiological processes by affecting the enzymes of phytohormone synthesis and inhibiting the nutrients and ion absorption by affecting plasma membrane permeability. Solubility of allelochemicals is one of the major factors determining their *phytotoxicity* activity (*23*).

Conclusion

E. gomphocephala leaf aqueous extract appeared to have an allelopathic effect on germination and seedling development of native *C.siliqua* seedling. The reduction was varied and could be extract concentration and season dependent. The highest allelopathic effects on germination and seedling development of native *C.siliqua* were recorded for samples are collected during the summer season. Generally, it is concluded that the allelopathic effect of introduced *E. gomphoracephala* trees on native *C. siliqua* trees has been weak or no effectiveness in some cases under laboratory conditions.

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