



Effect of Foliar Application of Plant Extracts on the Growth Behavior and Quality of Evening Primrose (*Oenothera biennis* L.)

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ABSTRACT

The indeterminate behavior of evening primrose is a frequent limitation in its cultivation. Controlling the final growth of plants at a certain stage is important. In the present experiment, different plant extracts were compared functionally with cycocel at different times of application. The experiment was performed as a factorial experiment based on a randomized complete block design with three replications. Treatments included normal water (control), cycocel (zero and 100 ppm), walnut (*Juglans regia* L.) leaf extract (zero and 25%), oak fruit extract (*Quercus castaneifolia* L.) (zero and 25%), and cotton capsule extract (*Gossypium hirsutum* L.) (zero and 25%). Evaluations were made on the seeds, oil yield and yield-components. The foliar application significantly reduced stem length (43-80%) and plant height (13-33%), but increased the percentage of oils (18.75%) and free fatty acids (67.5%). The highest oil content (35%) occurred in response to a combination of walnut, oak, cotton extracts, and cycocel. The highest amount of free fatty acids (23%) indicated more immature seeds and was obtained under the treatment of cotton extract. Natural extracts increased the oil percentage, but reduced the free fatty acid percentage and the stem length. It seems that further studies in this regard can help control non-terminal growth of evening primrose without having a need for breeding programs.

Introduction

Recently the proven effectiveness of medicinal plants, and increasing concerns regarding the side effects of chemical drugs on their long-term use, have augmented the use of medicinal plants and herbal medicines in different countries (Dattner, 2003; Mulat et al., 2020). Essential oils that are derived from plants have long been used as perfumes or flavorings in foods and beverages, as well as herbal medicines (Shibamoto et al., 2010; van Vuuren et al., 2009). With an average mature height of 30 cm, evening primrose (*Oenothera biennis* L.) is a biennial medicinal plant of the genus Onagraceae, and is native to North America. Yellow flower is an oilseed plant

with leaves that can be consumed orally (Gaertner, 1968; Wu et al., 2010; Sohrabi et al., 2017). The importance of this plant is the rare gamma linoleic acid content of its seeds (Ghasemnezhad and Honermeier, 2007). Essential fatty acids in the oil of evening primrose include linoleic acid (28 to 33%), gamma linolenic acid (7 to 10%) and their derivatives which also contain phenolic substances, flavonoids and tannins (Sohrabi et al., 2016) with several medicinal effects (Singh et al., 2012). There are several reasons that prevent evening primrose from cultivation in large scales. These problems include the biennial life cycle of the plant, indeterminate inflorescence and the high

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prevalence of seed shattering at ripening. Although the said issues are important goals in the specifics of plant breeding, it can be important to use farming methods so as to run on optimal costs and achieve acceptable outcomes with suitable cultivars. The effects of plant extracts on plant vegetative growth behavior have been described in the available literature (Amini et al., 2016; Kaur et al., 2004; Khosravi and Behzadi, 2006; Kocacaliskan et al., 2009). The leaf extract of *Atriplex canescens* and flower extract of *Achillea millefolium* negatively affected the radicle growth of lettuce seedlings (Amini et al., 2016). Moreover, studies have shown that the extracts of *Hypericum myrianthum* and *Hypericum polyanthemum* caused inhibition of lettuce growth (Fritz et al., 2007). Aqueous extracts of *Mikania glomerata* also reduced the percentage, fresh and dry weight and also length of tomato roots (*solanum lycopersicum*) (Ismail and Chong, 2002). Manzoor (2013) reported that the aqueous extract of *Calotropis procera* leaves had an inhibitory effect on lentil growth, whereas the extracts of *Eugenia jambolana*, *Nerium oleander* and *Citrullus colocynthis* improved the growth and yield of lupine plants (Abdel-Monaim et al., 2011). Soaking bean seeds in *Moringa oleifera* leaf extract increased growth and yield of bean plants (*Phaseolus vulgaris*) (Rady et al., 2013). In a relevant research, Valthiyanathan et al. (2014) concluded that the phytotoxic effect of the extract is a concentration-dependent phenomenon. Growth regulators are chemicals that alter growth and developmental processes, thereby increasing yield and seed quality, improving oil content, and facilitating the harvest of some plant species (Sohrabi et al., 2017a, b; Espindula et al., 2009). Cycocel is known in various formulations as “cycocel”, with the trademark name (CCC), and chemically serves as tri-methyl ammonium chloride (Waddington and Cartwright, 1982). By disrupting the pathway of the gibberellin biosynthesis cycle, cycocel inhibits the synthesis of ent-kauren synthase and reduces plant height by preventing cell elongation and growth (Rademacher et al., 2000; Khalilzadeh et al., 2016). It is reported that the use of cycocel at a rate of 2000 mg/l significantly increased the total chlorophyll content of *Zinnia elegans* (Rossini-Pinto et al., 2005). Also, experimental results on olive plants showed that cycocel treatments reduced seedling height, caused the elongation of roots and increased the number of lateral branches, compared to the control. A high concentration of cycocel (1000 mg/l) increased the fresh weight of leaves and chlorophyll content compared to control plants (Akbari and Jalili-Marandi 2013). In another

study, it was indicated that spraying CCC and Uniconazole (UN) on plants (as a plant regulator) inhibited the growth of zinnia plants in terms of height (Alami et al, 2020). Therefore, in the present study, different plant extracts were used as foliar applications to find an organic combination that could help control the “indeterminate” growth pattern of evening primrose, so that seed ripening could be synchronized to a reliable extent.

Materials and Methods

This experiment was carried out in two years (2016-2017) in the research farm of the Faculty of Agriculture, Gorgan University of Agricultural Sciences and Natural Resources, with a longitude of 54° 16', latitude of 36° 51' and an altitude of 13.3 meters above sea level, where the annual rainfall averaged at 607 mm. A factorial experiment was used with two factors as treatments: foliar application and time of foliar application, based on a randomized complete block design, with three replications. Due to the small amount of information about the effect of plant extracts on the growth behavior of evening primrose, the following concentrations were selected, based on the literature and the experience of authors. The treatments were, namely, 10 foliar application solutions including the control (i.e. normal water), cycocel at concentrations of zero and 100 ppm (Alami and Karimi, 2020), walnut leaf extract (*Juglans regia*) (zero and 25%), oak fruit extract (*Quercus castaneifolia*) (zero and 25%), cotton capsule extract (*Gossypium hirsutum*) (zero and 25%) and a combination of these treatments. As the second factor, the foliar sprays were applied at two stages. The first foliar application was performed when approximately 75% of the capsules had turned brown (Ghasemnezhad and Honermeier, 2007 and 2008). The second spraying was carried out two weeks after the first spraying. Seeds were collected from the plants in the research station. In order to provide the seedlings, the seeds were planted in cold conditions in November, and the seedlings were transplanted in the field in early spring. Before transplanting the seedlings, physicochemical characteristics of the soil were analyzed in the experimental site (Table 1). The dimensions of the plots were 1.5 m² with five planting rows in each plot and an inter-row distance of 20 cm. Weed management was done manually. At all stages of growth, the amount of irrigation was managed parallel to the amount required by the plant and according to climatic conditions.

Table 1. Soil physical and chemical properties of the experimental site

Soil type	Absorbable potassium (ppm)	Absorbable phosphorus (ppm)	Organic carbon (%)	Nitrogen (%)	Electrical conductivity (ds/m)	pH
Silty-loam	289	14.25	1.65	0.1	0.79	7.6

Preparation of plant aqueous extracts

In order to prepare the extracts of the plants (i.e. walnut leaves, oak fruit and cotton capsules), the soaking method was used, whereby the plant sample was first dried at room temperature, carefully powdered and mixed with distilled water at a ratio of 1: 10. The mixture was placed on a shaker for 48 hours at 100 rpm. After 48 hours, the desired composition was filtered and the plant extracts were stored in separate containers (Mansouri 2017). Some growth and yield indices were measured to evaluate the growth-reducing effect of plant extracts. Measurements were aimed at stem length, total green length, number of flowers, number of newly-formed flowers, number of seeds in each capsule, thousand seed weight, seed yield and the seed oil and free fatty acid percentage of oil.

The green part of each plant at each growth stage was measured by a ruler and was recorded in millimeters as total green length. The number of complete flowers in each plant was counted and recorded. The number of newly formed flowers was calculated similarly. At least 7 capsules from each plant were randomly selected and, subsequently, the number of seeds in each capsule was counted. The total number of seeds was divided by the number of selected capsules. To calculate the seed yield, the seeds of each treatment were separated and weighed.

Extraction of seed oil: extraction of evening primrose seed oil was performed by Soxhlet, with hexane solvent (Ghasemnezhad and Honermeier, 2007). Accordingly, in order to extract the oil, the powdered seeds were placed in a 105 ° C oven for three hours. Then, 5 g of the dried powder was placed on the cellulose fingers in the extractor part of the Soxhlet apparatus. The oil was extracted at 60 ° C for 8 hours. After oil extraction, desalination was performed in a dark oven at 37 ° C for 12 hours and the oil percentage was calculated (Da Porto et al., 2012).

Measuring free fatty acids: One gram of oil was added to the Erlenmeyer flask containing 50 ml of the solution of ethanol and toluene (1/1). Then, it was shaken to be dissolved. A few drops of Phenolphthalein solution were added to the mixture, and titration was performed by

potassium hydroxide (0.1 M, 100 µl) until a pink color appeared. The percentage of free fatty acids was calculated by the following formula:

$$\text{FFA} = (56/1 \times V \times C) \div G \text{ (Ghasemnezhad and Honermeier, 2007).}$$

Where, C: soluble molarity. G: weight of oil sample. V: volume of added chemical

Data analysis

Data analysis of variance was performed with SAS software (version 9.4) and mean comparisons were conducted using LSD test at 5% probability level. Graphs were also drawn with Microsoft Excel.

Results and discussion

Stem growth changes after foliar application

The results showed that the length of the formed stem was affected by the single effects of foliar application and time at the level of 1% probability. However, it was not affected by the interaction of the treatments (Table 3). Changes in stem length showed that the application of different foliar sprays reduced the stem length. The highest stem length (320 mm) was related to the control treatment and the lowest stem length (63 mm) was observed in plants affected by cycocel which caused a reduction of approximately 80% (Figure 1). Compared with the first foliar application (233 mm), the decrease in stem length by the second foliar application (99 mm) was approximately 58% (Figure 2). The use of growth inhibitory compounds, especially cycocel, in plant growth management has been previously studied and the results indicated an appropriate inhibitory effect which was caused by this compound. For instance, the foliar application of cycocel on *Erysimum marshallii* showed that the height of the treated plants was significantly reduced compared to the control (Bhat et al., 2011), which confirmed our results in the present study. Terzi (2008) in evaluation of the effect of walnut extract on germination and growth of melon and cucumber seedlings, reported that walnut leaf extract reduced root dry weight and stem length of melon and cucumber seedlings. In walnut leaves, Juglone reduced the growth of cucumbers and melons by stimulating

the synthesis of abscisic acid (ABA) and by preventing the production of growth hormones. Cycocel has also been shown to inhibit the synthesis of ent-kaurene synthase by disrupting the gibberellin biosynthesis cycle pathway and by reducing plant height, via the inhibition of cell elongation and growth (Rademacher et al., 2000; Khalilzadeh et al., 2016).

The analysis of variance (Table 3) showed that the vegetative growth of the plant was not affected by the interaction between the treatment and the time of treatment. However, the percentages of oils and free fatty acids were affected by the interaction of treatment type and time.

Table 3. Variance analysis of different foliar applications and their time on evening primrose (*enothera biennis* L.), growth and quality parameters

Sources of variation	df	The length of formed stem	Total green length	Number of flower per plant	Weight of single capsule contain seed	Seed number per capsule	Thousand seed weight	Seed yield	Seed oil percentage	Free fatty acid
Foliar application	9	6336.3**	404949.6**	747.4 ^{ns}	0.0006 ^{ns}	2170.1 ^{ns}	0.008 ^{ns}	19.38 ^{ns}	34.4**	76.59**
Time	1	292346.4**	528872**	5018.1**	0.02**	1026.7 ^{ns}	0.265**	43.30 ^{ns}	1.26 ^{ns}	698.3**
Foliar application × Time	9	18199.3 ^{ns}	29343.3 ^{ns}	155.6 ^{ns}	0.0001 ^{ns}	284.1 ^{ns}	0.014 ^{ns}	1.86 ^{ns}	5.22**	96.5**
Block		3985.5 ^{ns}	14946.1 ^{ns}	1024.1 ^{ns}	0.0008 ^{ns}	1974.5 ^{ns}	0.003 ^{ns}	30.23 ^{ns}	2.387 ^{ns}	0.655 ^{ns}
Error	38	24125.1	60476.6	582.8	0.0007	19.9.7	0.007	13.13	2.001	1.4
CV(%)		14.3	13.25	15.2	18.44	6.34	21.22	15.16	4.8	18.9

^{ns}, * and ** are non-significant, significant at the 5 and 1% levels, respectively.

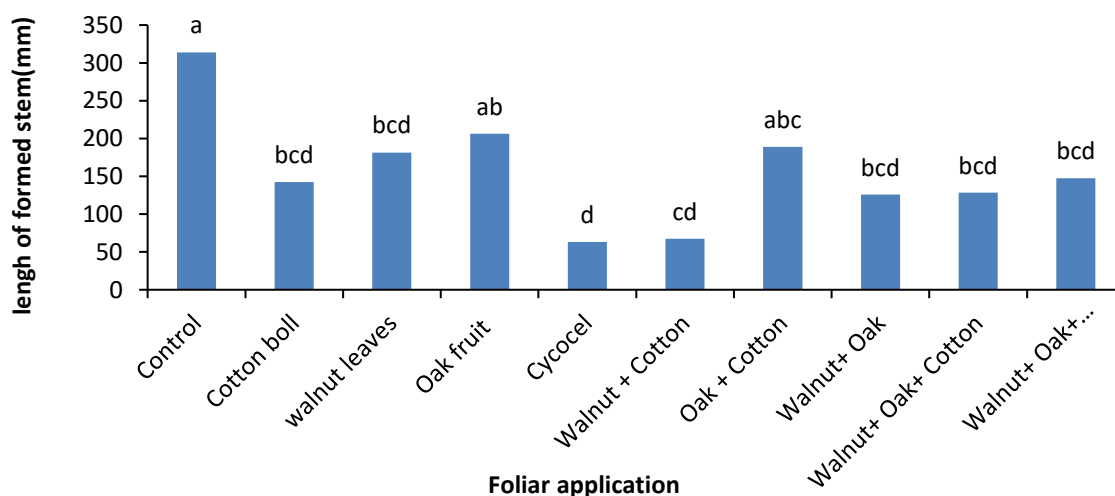


Fig. 1. Mean comparison of the effect of foliar application of different solutions on the stem length of evening primrose. Different letters indicate a significant difference at the level of 5%.

Mean value comparisons showed that the plant height decreased under the influence of all applied treatments. However, the intensity of this decrease after the treatment was affected by the type of treatment. As shown in Figure (1), the greatest reduction in growth was observed in plants treated with cycocel. However, the composition of walnut and cotton extracts was not significantly different in terms of the effects they caused. The effect of application time on

plant height showed that the second time of spraying had a greater effect on reducing the stem height (Figure 2).

Total green length

The analysis of variance showed that the single effects of foliar application and time were significant on plant height. However, their interaction had no significant effect on plant height (Table 3). The mean values of comparison

showed that foliar application reduced the green length of the plant. The foliar treatments in combination acted as an effective treatment to reduce the green part of the plant and reduced the growth of the green part as well. The maximum plant height (1263 mm) was observed in the control treatment in which no extract was used (Fig. 2).

Among the different concentrations of plant extracts, the aqueous extract (cotton capsule) showed a maximum reduction in plant height (843 mm) in the same statistical group that combined treatments of cycocel, oak + cotton, walnut + oak, walnut + oak + cotton (Figure 3). As shown in Figure (3), all applied treatments reduced plant growth in comparison with the control, although with variable effects. When plant extracts were used individually, no significant difference was observed between the

effects of cotton capsule and cycocel. Among the plant extracts, oak fruit was the least effective and, in contrast, walnut leaves were not significantly different from the two other groups. The dual combination of plant extracts showed a significant difference, however, while the combination of walnut and cotton extracts had a weaker effect on growth. Interestingly, the effect of cycocel on inhibiting the plant growth was reduced when cycocel was used in combination with other plant extracts (Figure 3). Also, mean value comparisons which related to the time of foliar application showed that the amount of new stem formation decreased, compared to the first time of foliar application, so that the maximum plant height (1020 mm) by foliar spraying at the first time was obtained and, in the second stage, it showed a decrease of 27% compared to the first stage (Figure 4).

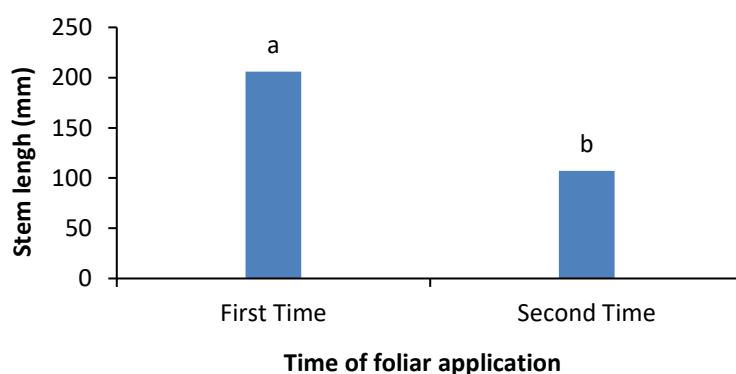


Fig. 2. Mean comparison of the effect of foliar application time on stem length of evening primrose (different letters indicate a significant difference at the level of 5%).

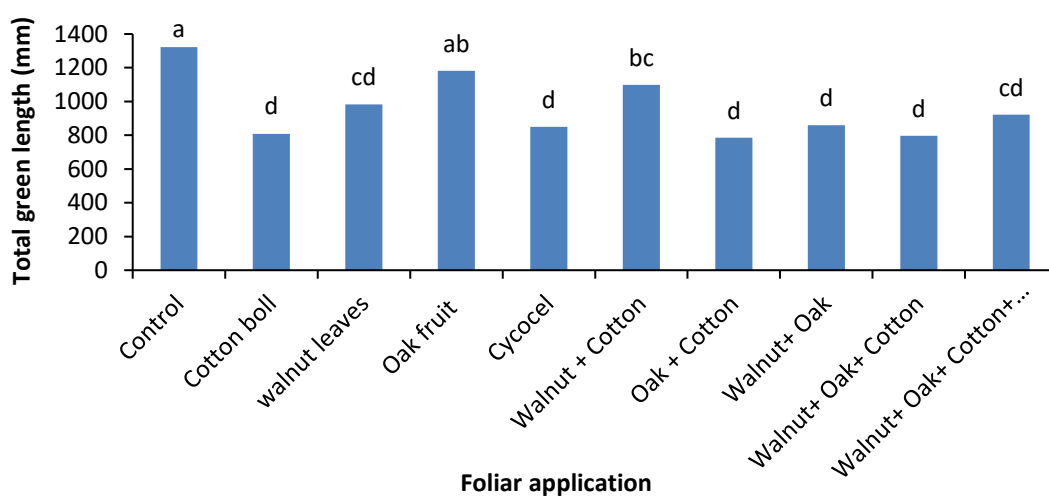


Fig. 3. Mean comparison of the effect of foliar application of different solutions on the total green length of evening primrose. Different letters indicate a significant difference at the level of 5%.

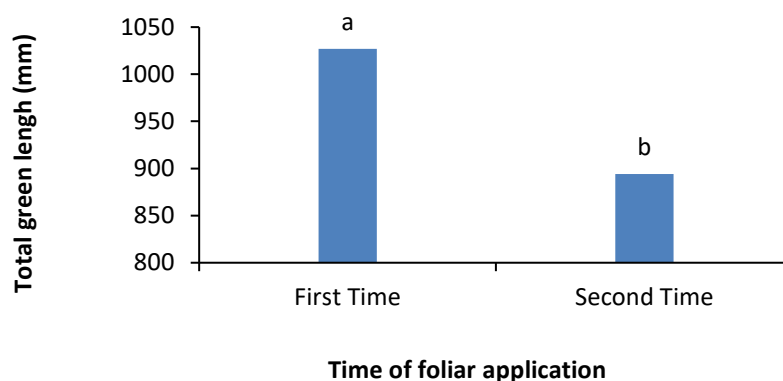


Fig. 4. Mean comparison of the effect of foliar application time on total green length of evening primrose. Different letters indicate a significant difference at the level of 5%.

The effect of plant allelopathic compounds on the growth and development of other plants has already been proven (Taiwo and Makinde, 2005). Specific growth-inhibitory compounds in walnut (Juglone) and cotton (Gossypol) decreased growth by hampering the process of photosynthesis and, especially, by causing changes in the hormonal ratio of the plant (Hejl et al., 1993; Hron et al., 1990). Previous studies have shown that these compounds inhibit plant growth by increasing the concentration of growth inhibitory hormone (ABA) in comparison to growth-promoting hormones such as auxin and cytokines (Jose and Gillespie, 1998). Khabbazi-por (2014) used a combination of paclobutrazol for 50 days on strawberries after planting, and concluded that the use of this combination had a different effect on the measured traits.

Paclobutrazol significantly reduced the occurrence of creeping stems and had no significant effect on leaf number and petiole length. Previously, it had been shown that compounds containing growth inhibitors can inhibit the biosynthesis of growth hormones, especially gibberellic acid, which is one of the main factors that increase vegetative growth in plants (Sohrabi et al., 2017; Khabbazi-por et al., 2014). Sandhya et al. (2014) studied the phytotoxic effects of aqueous extract of walnut leaves on the germination and early growth of mustard seedlings (*Brassica campestris* cv. 'Karanti'). They reported that the aqueous extract, relevant to concentration, inhibited germination and growth of the plant so that the highest toxicity occurred in response to the full concentration (100%) of the extract.

The number of flowers formed under the influence of type and time of treatment

The number of flowers per plant, as one of the effective factors, played an important role in plant yield. The data in Table 3 showed that the use of different concentrations of aqueous extract and cycocel did not significantly affect the number of flowers per plant. The effect of time, however, was significant on this trait at the 1% probability level. According to the results, there was a significant decrease (50%) in the number of flowers which formed during the second foliar application (Figure 5). It seems that delays in treatment application led to fewer flowers in formation. On the one hand, in addition to vegetative growth, foliar application was also effective on the reproductive phase. Considering the process of flowering induction, environmental and internal signaling for the formation of flower buds in plants, this process is done in a certain period of time. On the other hand, the availability of nutrients, specific and appropriate levels of plant hormones for each plant, at that time, helped induce flowering and flower formation, which explains the importance of foliar application time (Taiz et al., 2015). Considering the physiological effect of the treatments applied on the evening primrose and also the different growth conditions of the plant at the time of foliar application, it seems that at the first time of foliar application, the plant had a better physiological condition for growth and reproduction. In other words, the plant was younger. The first time of foliar application had the least negative effects on inducing flower buds in evening primrose flowers and on the number of flowers. The effect of growth regulators on

flowering has long been proven in plants, so far that it has become commonplace in the management of fruit trees and fruit yield in modern orchards (Manu et al., 2020; Engin et al., 2019). In the jujube tree (*Ziziphus mauritiana*), the use of foliar application with growth regulators at different times caused a significant increase in the number, size and weight of fruits (Nakhaee, 2016) which turned out as consistent with our results about the effect of foliar application time.

Changes in single capsule seed weight

According to the analysis of variance (Table 3), among the treatments, only the spraying time at 1% had a significant effect on single capsule seed weight. The mean value of this trait showed that the single capsule with seeds was heavier (0.11 g) in the second time of foliar application, compared to the first time of application (Figure 6).

A delay in foliar application reduced the number of flowers per plant, which enhanced capsule weight. Considering the effects of foliar application time on the number of flowers per plant, the results indicated more flower formation in the first time, and the increase in single capsule weight under the influence of the second foliar application time was anticipated due to the smaller number of capsules. Fewer flowers and fewer capsules resulted in larger capsules with more seeds. In fact, this point was mentioned in a study by Pouryousef and Shahi (2016) in the safflower plant, which indicated that under the influence of spraying time, grain weight was significantly affected in pods (Pouryousef and Shahi, 2016).

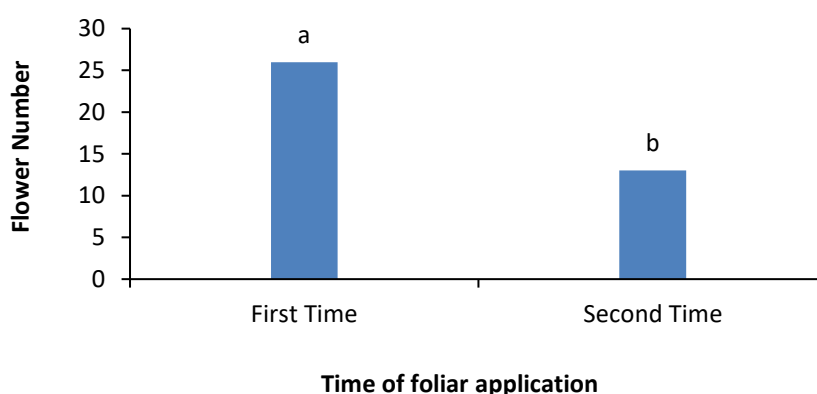


Fig. 5. Mean comparison of the effect of Foliar application time on flower number of evening primrose (different letters indicate a significant difference at the level of 5%)

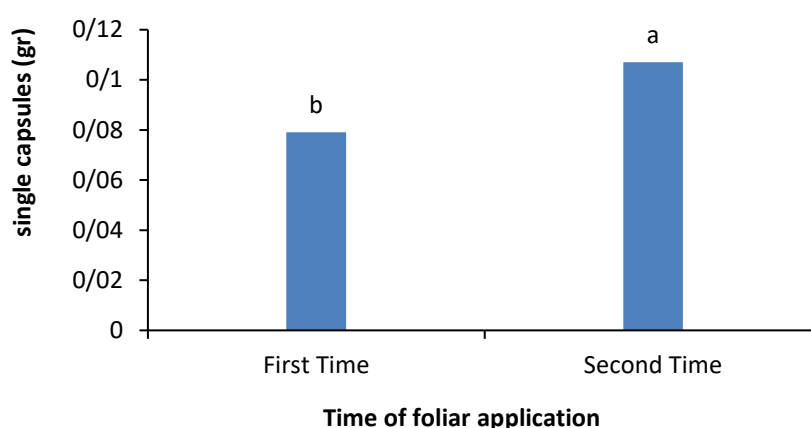


Fig. 6. Mean comparison of foliar application time on single capsules containing seeds of evening primrose. Different letters indicate a significant difference at the level of 5%.

Thousand-seed weight, number of seeds per capsule and seed yield

According to variance analysis (Table 3), the effect of treatment and the interaction of treatment and time on the 1000-seed weight were not significant. However, under the influence of foliar application time, the 1000-grain weight was significantly different at 1%. Mean value comparisons of the two spraying times suggested that the highest 1000-seed weight (0.45 g) was obtained in the second spraying time, and became approximately 22% higher than that of the control (Figure 7). As one of the most important yield factors, grain weight is partly shaped by the availability of photosynthetic products, especially in the early stages of grain growth, and consequently to the number of growing seeds that save photosynthetic products. One of the ways to achieve high yield in plants is higher allocation of photosynthetic materials for seeds (Felekari et al., 2014). Although grain weight is a genetic trait that is less affected by environmental factors (Shah et al., 2017), sometimes different nutritional behaviors affect grain weight. Foliar application time is an important factor in various plant parameters. A decrease in the number of flowers, due to foliar application, makes the food

source superior to the sink and, thus, increases the weight of seeds in each capsule. In fact, by reducing the number of flowers, the number of seeds as a reservoir of plant consumption decreases and more nutrients are accumulated per seed. Such an increase in nutrients will augment the weight of seeds (Saeedi et al., 2011). The number of seeds per capsule and seed yield were not affected by the experimental treatments.

Oil percentages

The effect of foliar application type, foliar application time and their interaction on evening primrose oil (%) showed that the percentage of seed oil was affected by foliar application, foliar application time and the interaction effect of treatments (Table 3). Mean value comparison of foliar application type and time interaction showed that the highest percentage of total oil (32%) was observed in response to the second time and with using plant extracts of walnut leaf, oak fruit, cotton capsule and cycocel. The lowest percentage of oil (26%) was recorded in the second time with cycocel treatment. Compared to the control, applying plant extracts in the second time increased the oil accumulation by 25% (Figure 8).

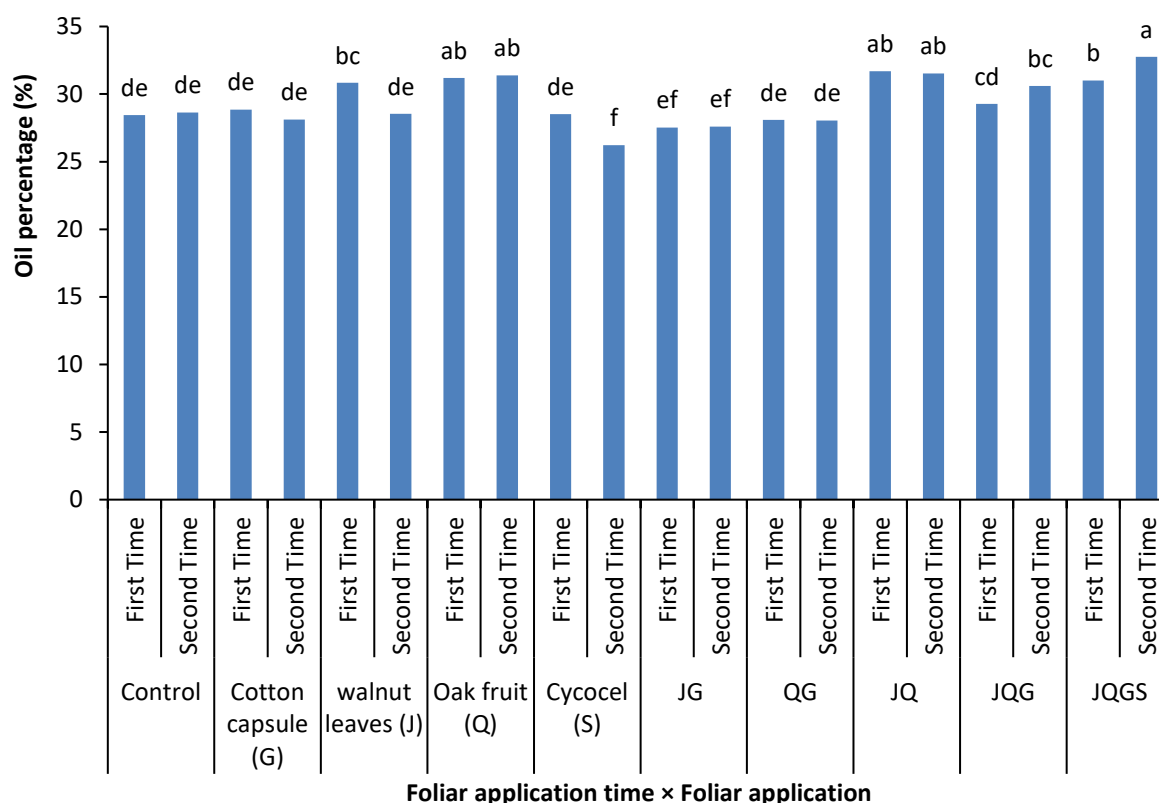


Fig. 8. Mean comparison of the effect of foliar application time and type on seed oil percentage of evening primrose. Different letters indicate a significant difference at the level of 5%.

Researchers have reported that the use of foliar application with cycocel, Pix, and Alar growth regulators increased cotton seed oil content (Sawan et al., 2001).

Free fatty acids

The analysis of variance showed that the effects of foliar application type and time, as well as their interactions, were significant on free fatty acids content at the level of 1% (Table 3). According to the mean value comparison of effects, the highest and lowest amount of free fatty acids were obtained under the influence of cotton capsule extract (23%) and by spraying the cycocel solution for the second time, respectively (Figure 9). The percentage of free fatty acids in the oil of a seed plays a key role in determining its quality. A higher free fatty acid content in the oil implies

oil instability and a greater likelihood of spoilage, as well as the formation of harmful compounds (Malik, 2019). The seed oil of evening primrose contained glycerol, mono-glyceride, di-glyceride, tri-glyceride and some free fatty acids. A high percentage of free fatty acids in seed oil at harvest time means that the seed is not yet mature and that the formation of oil is not complete, primarily because of a lack of attachment of fatty acids to the glycerol skeleton through esterification. In contrast, a high fatty acids content in seed oil after storage can result from distortion and oil spoilage (Malik, 2019). However, as mentioned, the presence of free fatty acids above the standard amount can be a sign of low oil quality. High levels of free fatty acids under the influence of some treatments, such as cotton capsules, can result from various causes.

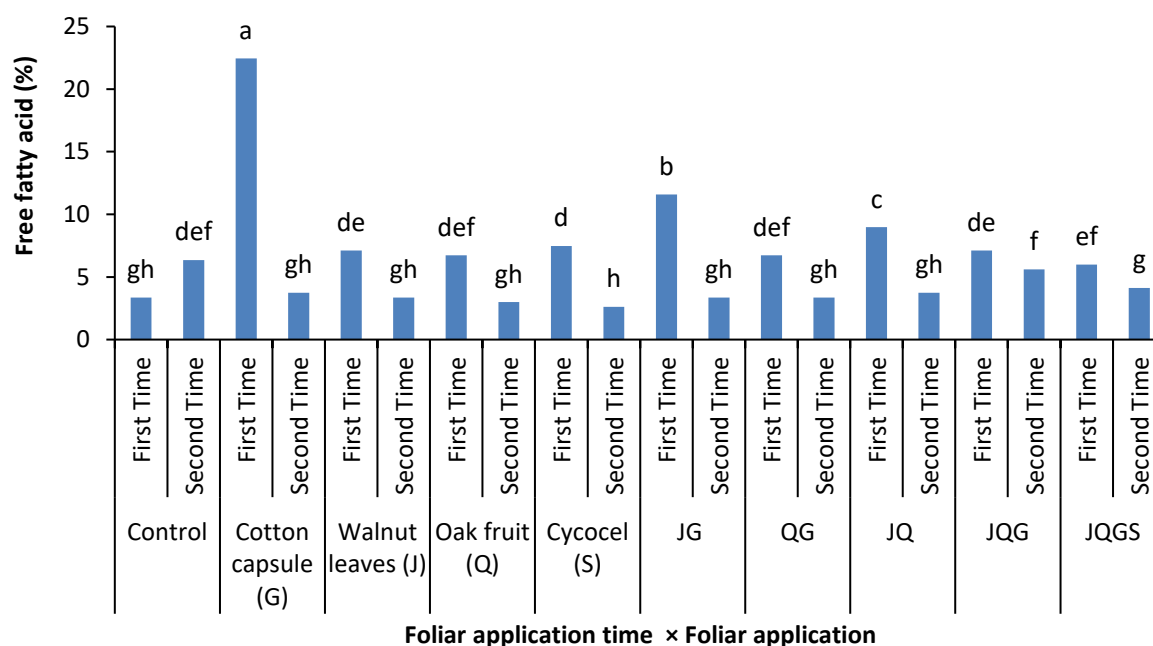


Fig. 9. Mean comparison of the effect of foliar application time and type on the free fatty acid content of evening primrose. Different letters indicate a significant difference at the level of 5%.

Conclusion

In the present study, the effects of foliar application of walnut, oak and cotton plant extracts, as well as cycocel, as a growth regulator, were evaluated with the aim of reducing vegetative growth and avoiding the reproductive growth of evening primrose. According to the results, the use of cycocel significantly reduced the growth parameters, whereas the plant extracts increased the percentage of oil and fatty acids compared to the control. Considering the inhibitory effects of the applied plant extracts on the vegetative and reproductive growth of evening primrose, compared with the control and cycocel treatments, it is suggested that the timing of treatments should be considered more carefully for future research perspectives on this issue.

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Conflict of interest

The authors indicate no conflict of interest in the present work.

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