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Original Article

Effects of some Ggronomic Techniques on Oil Yield and Composition of *Oenothera biennis* L.

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Abstract

Evening Primrose oil (*Oenothera biennis* L.) is a rich source of Gamma linolenic acid (GLA) and other useful unsaturated fatty acids. Despite the diversity of sources of GLA, due to better absorption in the human body, evening primrose oil is the main source of GLA. In this study, the effects of pollination, harvesting method and application of salicylic acid on yield, yield components, and quality of evening primrose oil were studied. A factorial experiment as a randomized complete block design with three treatments including pollination (pollination by bees, no pollinators and free pollinators), salicylic acid (0 and 200 ppm) and harvest method (normal harvest, pre harvest by flame) and four replications was performed. The effect of treatments on the oil yield, oil content, fatty acids composition and the content of free fatty acids of oil were studied. Results showed that the oil yield and oil percentage of plants which visited by bees were at the highest amount (1.87 gr/ plant and 33.6%, respectively). Compared to the control, the oil yield of plants which treated with salicylic acid significantly increased. Between two different harvest methods, the highest oil percent (31.6 %) was observed in plants treated with flame. Under the interaction effect of treatments, the highest oil yield (2.24 g/plant) was observed in combination of bee and salicylic acid 200 ppm and the highest free fatty acids (2.19%) accumulation was recorded in the open cage with 200 ppm salicylic acid. Since pollination increases the number of formed seeds per capsules, therefore, it is expected that simultaneous application of pollinator and pre-harvest treatment by flame increases seed yield, oil yield and quality which is partly shown in the obtained results of present study.

Keywords: Salicylic acid, Harvesting method, Pollinators, Seed yield, Oil quality

Introduction

Evening primrose (*Oenothera biennis* L.) is a biennial plant of the family Onagraceae and *Oenothera* L. genus [1]. Evening primrose oil is a rich source of gamma linolenic acid (GLA) [2-4], and eczema, heart disease, facial acne, diabetes and cancer [5-8]. Despite the various sources of gamma-linolenic acid, especially in Saxifragaceae, Boraginaceae and Onagraceae [9], more specific and biological compatibility with the human physiology, evening primrose oil is the best source of acid GLA [10]. Salicylic acid (SA), ortho hydroxy benzoic acid, with a phenol compound is in the category of natural plant hormones [11]. Stomata opening and closing, seed germination, uptake and transport of ions, photosynthesis, stomata conductance, respiration, ripening, glycolysis, flowering and heat production, message carrier, resistance against abiotic and biotic stress are the most important role of SA in plants [12,13]. About the effect of salicylic acid on the yield and quality of oil in oilseeds [14], essential oil plants [15-17], several reports have been published. Salicylic acid as a compound involved in the resistance mechanisms and response of plants to

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stress is important [18-19]. It has been showed that salicylic acid as an elicitor induces expression of antioxidant enzymes like phenyl alanine amonialyas and stimulation the formation of osmoprotectants such as proline [20-23]. Proper performance and maximum active ingredients, is the breeding goals of a medicinal plant. Due to the long process of a variety program, agronomical practices that can increase the performance of the active ingredients in the plants are important. Indeterminate inflorescence, non-simultaneous ripening of seed and seed shattering during ripeness are the main barrier in front of the evening primrose becoming a commercial product [24]. Drying the leaves prior to harvest with herbicides and other methods are recommended in plants with asynchronous ripening [25]. Most plants directly depend on pollinators to agents for seed production [26]. Review of past research suggests that the presence of pollinating bees on the farm increased seed yield, seed weight and accelerate oil accumulation in rapeseed [27]. Research conducted at the plant Black seed oil (*Guizotia abyssinica* Cass) showed that the use of bees (*Apis cerana indica*) for black seed pollination increased the seed oil considerably [28]. In an experiment to investigate the effect of pollinators on yield and quality of oil of Jatropha (*Jatropha curcas* L), it was demonstrated that compared to self-pollination the oil yield increased when honey bee was used [29]. Based on the effect of pollinator not only the oil quantity, but also the fatty acids composition of oil also changed. The present research was aimed to study the effect of foliage application of salicylic acid, the method of harvest and pollinator on the quantity and quality of evening primrose oil has been designed and implemented.

Material and Method

Condition of Sowing the Seeds

Seeds used were prepared from research station of Gorgan University of Agricultural Sciences and Natural Resources. At 6-4 leaf stage on February 2015 Seedling were transferred in the pot with the medium composition of local soil: leaf composts: perlite (1: 1: 1) with electrical conductivity of 2.1 ds and pH=6.7.

Experiment Design and Applying Treatments

A factorial experiment based on the randomized complete block design with three treatments and four replications was performed. Pollination treatment was done in three levels of pollination by bees, no pollination using isolated cage and open pollination. Experimental plants were treated with Salicylic acid on two levels of 0 and 200 ppm. Based on harvest method treatments plants were harvested in two different forms. 1- one week before harvest the crown of the plants was burned with flame, 2-plants were harvested and dried under the field conditions. After the appearance of the first buds of flowers, all blocks except the block of free pollination were isolated by mosquito net. In all these steps irrigation was done according to the plant needs and the weather conditions. In pollination treatment a small size hive (population 700 bees) was used. Foliar application of salicylic acid was done in two stages within a week. SA treatment was done when one-third of capsules were brown. When 2/3 capsules were brown, in order to accelerate seed ripening the crown of plants were treated with flame. In this experiment, the effect of treatments on oil percent, oil yield, oil fatty acids composition and free fatty acids content were studied.

Sampling

For analyzing oil yield and composition of (*Oenothera biennis* L.), we weighted 5 g mature seed and dried and powdered with mixer.

Method of Oil Extraction

Seed oil was extracted by Soxhlet apparatus [30]. Wight of dry seed was 5 g and the solvent was Hexane. The time for extraction was 8 hour.

Method of Methylated Oil

The fatty acid composition of oil was analyzed by GC according to Kurt [31]. In order to analyze the fatty acid composition of seed oil, 0.3 grams of finely powdered seed was added in a 10 mm tube containing 3 mL of diethyl ether. The sample was refrigerated for 30 minutes and centrifuged at 4000 rpm for ten minutes. To remove the solvent, samples were placed in desiccator for 1 hour. Then 2 ml of sodium methylate were added to the mixture and was kept in dark place for 30 minutes. By adding one milliliter of isooctane to the mixture and after the formation of two phases, the upper phase was used for GC analysis.

Gas Chromatography Conditions

For the analysis of oil samples GC model Varian CP3800 equipped with an internal diameter of 25/0 mm Permabond® FFAP length of 25 m and CP 3800 was used. Injection temperature was 280 ° C with and 2 ml/M flow rate.

Formula and Data Analysis

Free fatty acid determination was done by titration according to the following formula: FFA= (56/1 × V × C)÷G. where V=titration value, C: solution Molarity, G: oil weight. In all cases, 5 grams of powdered seed was used for oil extraction. For data analysis SPSS. 20, software was used. The mean value was compared using least significant difference (LSD) test.

Results and discussion

Seed Oil Yield

The results of data analysis showed that SA and pollinator had significant effect on the oil yield at 1% (01/0 < p). Oil yield is also influenced by the interaction between pollinator and salicylic acid. The combined effect of salicylic acid and flame on seed oil yield was significant (Table 1). The interaction among treatments significantly influenced on seed oil yield. Mean comparison showed that the highest oil yield (1.9g) and the lowest oil yield (1.33 g) were observed in pollinated and non-pollinated plants, (Figure 1).

The results showed that under the effect of foliar application of salicylic acid, the oil yield of plant significantly increased. In comparison to the control plants, 0.27 grams oil per plant was increased (1.42 g/plant oil and 1.69 g/plant oil in control plant and plant which were treated by SA, respectively).

Simultaneous use of salicylic acid and pollinators (bees) significantly increased oil yield. A comparison of average data shows that the highest oil yield (2.24 g) in the treatment of bees and salicylic acid (Fig. 3). Variance analysis of data shows that the interaction effect of salicylic acid and the flame on the oil yield at the level of 5% is significant (Table 1). When flame and salicylic acid were used to accelerate the ripening of seed, an antagonistic effect was observed on oil production and plants which were treated with flame produced low oil. However, in the absence of salicylic acid the oil yield was higher in plants treated with flame (Fig. 4). Under the simultaneous application of three treatments, the highest oil yield was observed in plants that were pollinated by bees, were sprayed with salicylic acid and harvested manually (Fig. 5). Analysis of variance showed that the oil content of seeds was significantly influenced by the pollinator, harvest method and salicylic acid application. Despite that, no significant interaction effect of applied treatments on oil percent was observed among used treatments (Table 1).

Table 1: Variance analysis of the effect of pollinator, salicylic acid and harvest method on the oil yield and free fatty acid content of evening primrose seed oil.

	Mean Square		
Sources Change	Oil yield	Oil (%)	FFA
Bee	0.95^{**}	120.74^{**}	0.11^{ns}
SA	0.67^{**}	101.74^{*}	0.38^{*}
FL	0.0004^{ns}	82.08^{*}	0.24^{ns}
Bee × SA	0.48^{**}	39.85^{ns}	0.45^{*}
Bee × FA	0.19^{ns}	12.21^{ns}	0.09^{ns}
FA × SA	0.47^{*}	20.61^{ns}	0.46^{*}
Bee × SA × FA	0.32^{*}	24.72^{ns}	0.26^{ns}
Error	0.06	13.89	0.08

Bee: honey bee, SA: Salicylic acid, FL: flame.
^{ns}: no significant difference, * and ** significant at level five and one percent, respectively.

The highest oil percent was observed plants which had pollinator (bee blocks). Opposite to that, between isolated plants and plants under free pollination conditions no significant difference in oil percentage was observed (Fig. 6). Foliar application of salicylic acid in a concentration of 200 ppm was significantly increased the oil accumulation of seeds compared with control, 28.34 % compared to 31.79 % oil in without and with salicylic acid application, respectively (Fig. 7).

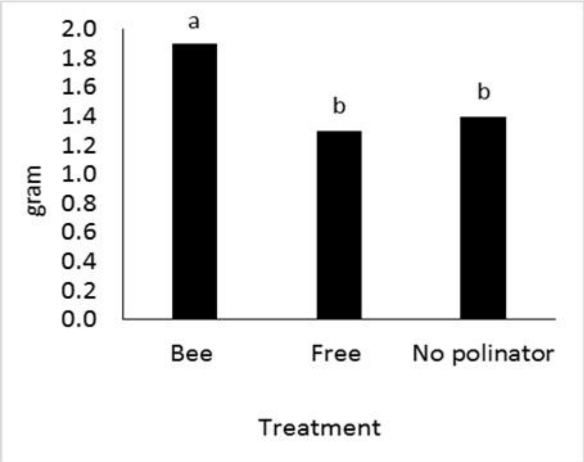


Fig. 1 The effect of pollinators on the performance of oil.

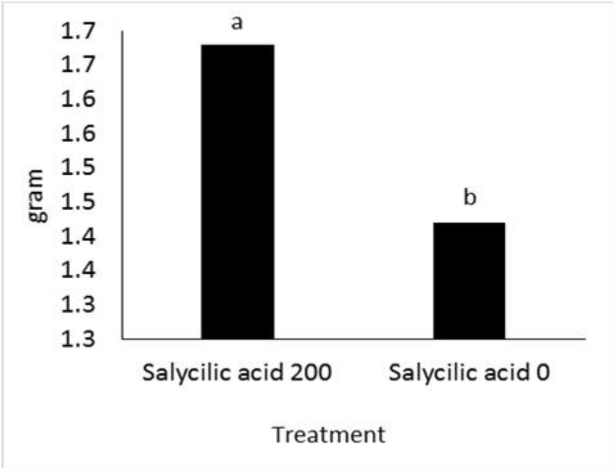


Fig. 2 Effect of salicylic acid on Oil.

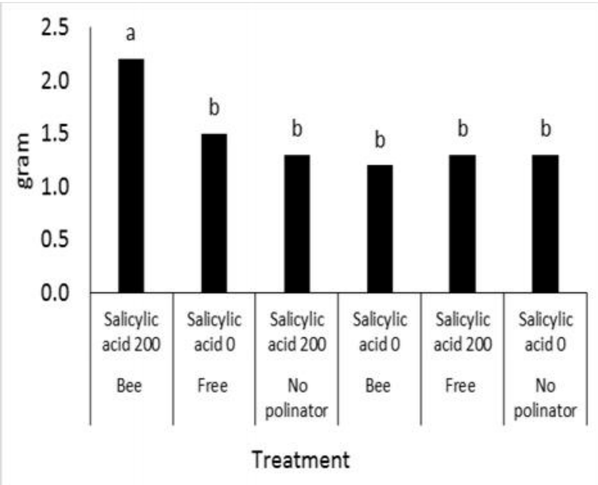


Fig. 3 Interaction effect pollinator and SA on the oil yield (5 g seed)

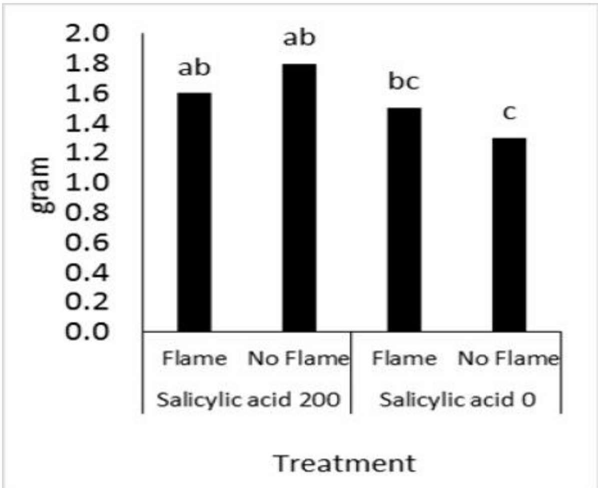


Fig. 4 Interaction effect of flame and SA on the oil yield (5 g seed).

Table 2 Analysis variance of the effect of pollinators, harvesting method and salicylic acid on the fatty acids composition of evening primrose oil

Sources of changes	df	palmitic acid	stearic acid	oleic acid	linolenic acid	GLA
Treatment	5	0.05^{*}	0.03^{*}	0.83^{ns}	1.42^{ns}	0.13^{ns}
Error		0.03	0.5	3.61	1.55	0.12

^{ns}: no significant difference, * and ** significant at level five and one percent, respectively.

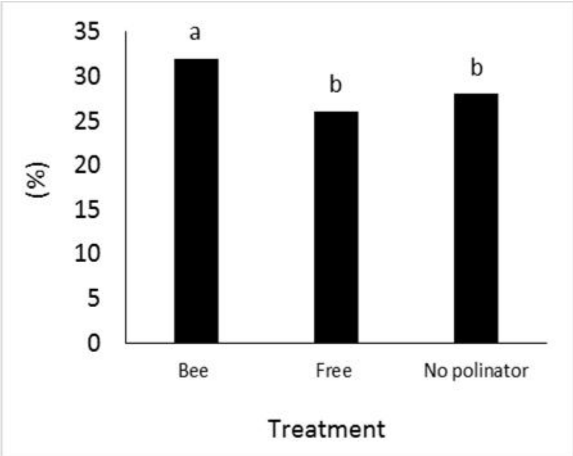


Fig. 6 The effect of pollinators on oil percent.

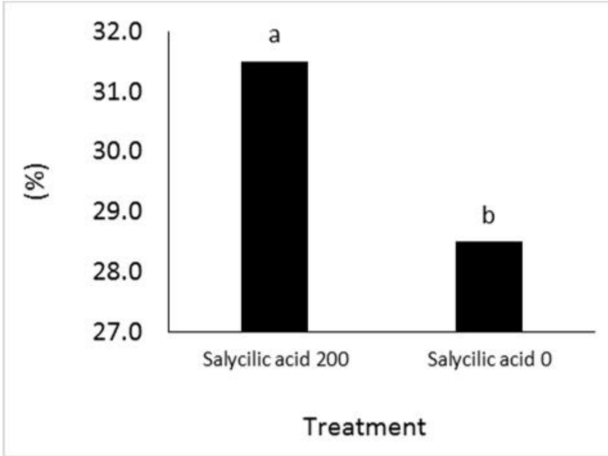


Fig. 7 Effect of salicylic acid on oil percent.

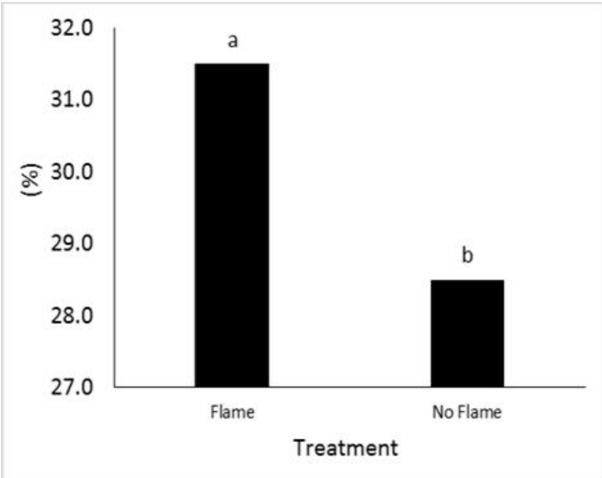


Fig.8 The effect of flame on oil percent.

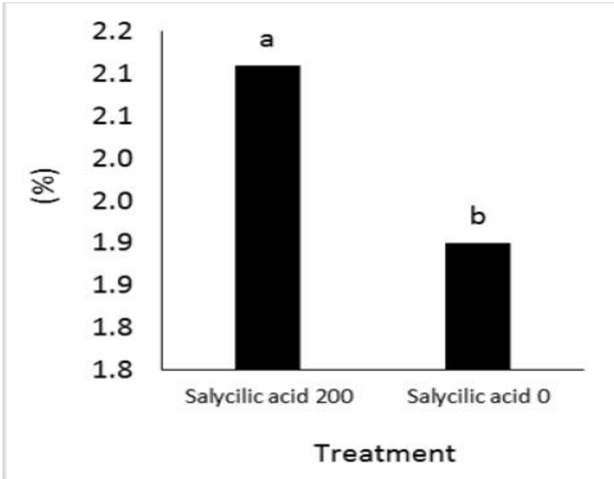
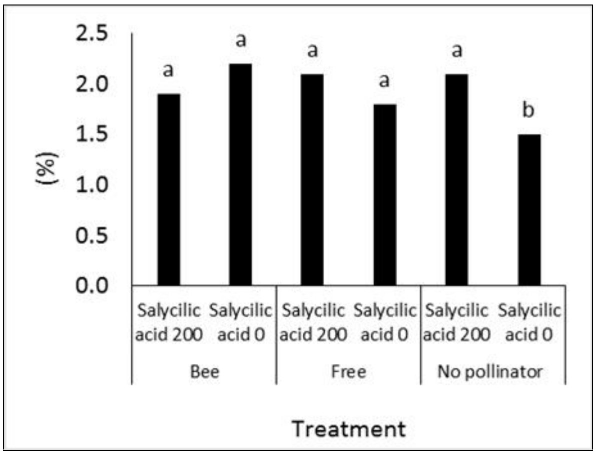


Fig. 9 Effect of salicylic acid on oil content.



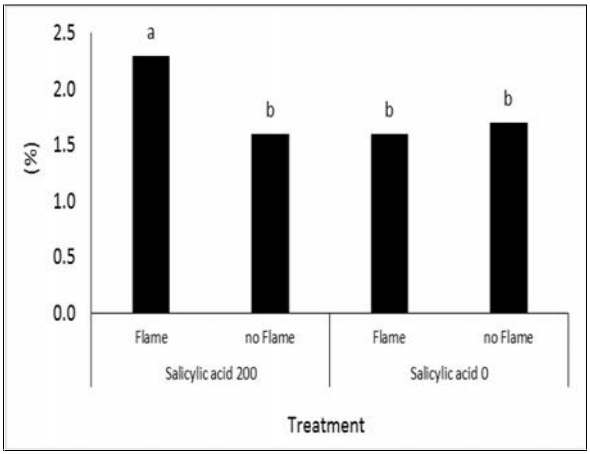


Fig. 10 Interaction of salicylic acid and bee on oil percent. **Fig. 11** Interaction effect of flame and Salicylic acid on oil free fatty acid percent.

Table 3 Comparison of the effect of pollinators (P), salicylic acid (SA) and harvesting (HM) on fatty acids in evening primrose oil 5 grams of seed

Treatments	palmitic acid (%)	stearic acid (%)	oleic acid (%)	linolenic acid (%)	GLA (%)
P0×SA1×HM1	8.03 b	5.84 a	25.63 a	56.65 a	6.65 a
P0×SA0×HM0	8.17 ab	5.09 b	25.76	56.97 a	6.65 a
P1×SA1×HM1	8.24 ab	5.84 a	24.63 a	57.12 a	6.7 a
P1×SA0×HM0	8.14 ab	5.93 a	25.27 a	56.87 a	6.59 a
P2×SA1×HM1	8.43 a	5.57 a	25.08 a	58.2 a	7.12 a
P2×SA0×HM0	8.17 ab	5.93 a	26.11 a	56.12 a	6.51 a

ns: No significant difference, * and ** significant at the level of five and one percent, respectively. P =: pollinators, P0 =open pollination, P1 =pollination with bees, P2 =without the pollinating; SA = Salicylic acid, SA0 =No salicylic, SA1 = 200 ppm salicylic acid; HM =Harvesting: HM0 = No pre harvest, HM1 = pre harvest treatment with flame.

Flame pre-treatment before harvesting to facilitate the harvest and the accumulation of seed oil showed that compared to untreated plants seed oil accumulation significantly increased (Fig. 8).

Fatty acids composition

The results of data analysis showed that evening primrose oil fatty acids composition was not affected by used treatments. As is clear from Table 2, only stearic acid as a saturated fatty acid was significantly affected by treatments. However, mean comparison of the data showed a significant difference in the contents of palmitic and stearic acids affected by treatments (Table 3).

Free Fatty Acids Content

Results of oil analysis showed that the free fatty acid content of the evening primrose seed oil was significantly influenced by used experimental treatments including pollinators, salicylic acid and harvest method. In this regard and compared to the control plants salicylic acid ($p < 0.5$), dual bee and salicylic acid ($p < 0.5$) and salicylic acid and flame ($p < 0.5$) interactions significantly influenced the difference free fatty acid content of oil (Table 1). The highest (2.11%) and the lowest (1.9 %) percentage of free fatty acid of oil were observed under 200 ppm salicylic acid (Figure 9). The interaction effect between pollinators and salicylic acid on the free fatty acid percentage of oil was significant. However, the lowest percentage of saturated fatty acids (1.59%) was observed in the combined treatments of open pollination and without salicylic acid applying (Figure 10).

Under the interaction effect of flame and salicylic acid, the highest percentage of free fatty acids (2.31 %) was observed in plants which were treated with 200 ppm of salicylic acid and treated with flame (Figure 11).

Discussion

The results showed that the highest yield of oil was recorded from plants grown in blocks with bees. In contrast, plants grown in a block without pollinators had the lowest oil yield. However, there was no significant difference between the isolated block and the one with open pollination conditions. The results of this study is in accordance with the findings of Negussie and his co-workers [29] who showed that the oil yield of Jatropha plant seeds increased at the presence of pollinators. The amount of oil in oilseed is affected by factors such as water stress, temperature, disease, nitrogen availability and etc. [24]. The researchers are of the opinion that in oilseed crops, there is a positive correlation between oil yield and seed yield [32]. Fertilization via pollination even in Cross-pollination plants or self-pollination plants plays an important role in seed yield increasing. In this regard it has been showed that, the yield of oilseed crops will increase under the effects of pollination [33]. This fact is obvious that successful pollination using pollinators increases the seed yield. In this regard, the results showed that the presence of pollinating bees on the farm increased grain yield, grain weight, seed quickly as well as the oil yield [34]. Mechanisms of pollination to increase the oil yield are definitely associated with hormonal changes (auxin and gibberellic) which induced in the plant by pollination [35,36]. The role of salicylic acid in the accumulation of seed storage compounds is not clear. But what is certain is that, this hormone-like compound through the induction of plant suspended tension forced it to react to stress. The reaction which is accompanied with the rapid growth and increasing food assimilation and transfer to the vital parts of the plant such as seeds is related. The finding of present research showed that salicylic acid significantly influences the oil accumulation of evening primrose. The same was reported by Athari and Talebi [14] in sesame. Under the water stress conditions, application salicylic acid in moderate level increased the oil accumulation to 49.27%. In contrast to that, Farjam *et al*., [37] has been reported that the oil accumulation of spring safflower seeds did not influence by salicylic acid application. It means that the influence of salicylic acid in seed oil accumulation could be related to the various factors including, plant species, plant grow behavior, and the stage of application. It was reported that, the salicylic acid improves plant growth the activation of enzymes involved in photosynthesis [38]. Salicylic acid has also been shown that by increasing photosynthesis and photosynthetic production and subsequent storage of these materials in seed, increased seed weight and seed yield [11]. Since the formation of oil in oil seed plants is related to the photosynthetic activity, thus the increased oil formation using foliar application of salicylic acid is explainable. Salicylic acid in low concentrations as stimulating plant growth and plant involved in removing oxidative damage. If used in high concentrations, induces plant stress [39]. In surveys conducted in Sunflower, it was shown that inducing stress to the plant during grain filling stage increased the oil content of seed. This is while, apply the same level of stress at flowering stage sharply increased the protein content of seed instead of oil has risen [40]. A negative relationship is between the protein content of the seeds and the oil quality of oil seed crops [24]. In a research on the effect of drought stress and salicylic acid application on the photosynthetic pigments and nutrients of sunflower, it was found that foliar application of salicylic acid (100 mM), had significant effect on the absorption of phosphorus as a source of transportation energy [41]. An increase in the absorption of phosphorus by application of salicylic acid equal to the increasing in internal energy transferring which resulted to the more seed oil is accumulation, could be another reason of more oil accumulation by using salicylic acid. From the obtained results it can be indicated that the using agricultural inputs improve the performance of evening primrose seed and oil yield. By burning plants to harvest from Crown, flow and vascular activity stop. In this case, the plant spends all his energy to transfer sugar and other nutrition able materials to the grains which are filling up. Degradation of phloem due to the burning of the skin, while the xylem is still alive and transfer minerals and water from the roots to the leaves, leads all assimilates to the grain and accelerate its filling. Accordingly, the flamed plants are not only more mature, but the seeds are more uniform. Palmitic acid and stearic acid are saturated fatty acids which produced prior to unsaturated fatty acids in fatty acid production. Observed significant difference in the contents of saturated fatty acids affected by used treatments in present research means that most cases the harvested seed samples contained high value of immature seed. That could be the reason why unsaturated fatty acid did not influenced by treatments. Unsaturated fatty acids play an important role in increasing the quality of vegetable oil. There is a direct relationship between the concentration of unsaturated fatty acids of the oil and seed maturity. Unsaturated lipid peroxidation of cell membranes in plants is one of the processes of coping with stress [42]. It was shown that under stress conditions, the amount of saturated fatty acids such as stearic acid, palmitic acid increased, while the levels of unsaturated fatty acids decreased [43-44]. It was also shown that foliar application of salicylic acid change compared to oil fatty acids [44]. Increasing the amount of unsaturated fatty acids such as linolenic, linoleic and oleic acids could be due to increasing the activity of enzymes delta-6 desaturase under the influence of salicylic acid which increases the membrane lipid stability and permeability [44]. The results of this study were consistent with the findings of Moradkhani and colleagues. Generally vegetable oil is a mixture of glycerol, mono glycerol, diaglycerol, triaglycerol and free fatty acids. When the seeds have sufficient time, under the influence of consecutive esterification reaction the number of triaglycerol will increase. This means that the time to harvest after seed set is longer and results more mature seed with higher oil yield. In other words, the lower the percentage of free fatty acids and the higher glycerides especially triglycerides means higher oil quality. Burn the crown, puts the plant under stress. In the short time remaining grain filling increased speed and increased formation of fatty acids and glycerol. However, there is ample opportunity for esterification reaction. It consists of a large number of fatty acids have the opportunity to hang the skeleton glycerol and remain in the free form in oil. In other words, the free fatty acids seed oil is higher; it means that the more immature seeds are harvested. It should be noted that free fatty acids in the process of corruption oil during storage quite different from that category. That is why under the effect of flame, despite an increase in the oil quantity due to high free fatty acids, the extracted oil is prone to oxidation and its quality is low. Therefore, it seems that the treatments applied to crops seeds provide valuable context for further maturation and should be taken into consideration. This point, in plants such as evening primrose with indeterminate inflorescence is more important.

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