IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 11, Issue 2 Ver. II (February 2018), PP 53-58
www.iosrjournals.org

Oil Content, Vegetative and Reproductive Traits of Jojoba Plant As Affected By Foliar Application of Gibberellic Acid

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Abstract: *This experimental was conducted to study the response of jojoba tree (Simmondsia chinensis) to foliar application of gibberellic acid (0,50,100,150 ppm) on vegetative, reproductive traits and the seed oil content at privet farm in EL-Behira governorate, Egypt during two successive seasons of 2016 and 2017. The experiment was arranged in Randomized Complete Block Design (RCBD) with three replications .Results revealed that all treatments significantly enhanced vegetative growth characteristics (branch length, secondary branches length, number of branched nodes and reproductive characteristics (flowering date, initial fruit set, final fruit set, seed yield and chemical composition of jojoba seed compared to the control in the first and second seasons, respectively, so seed oil fatty acids were identified . The treatment of 150ppm GA_3 gave the minimum 50% flowering (41.24 and 37.50) days and recorded the highest means of branch length (95.09 and 98.68), secondary branches length (50.29 and 52.84) and maximum final fruit set (91.05 and 91.23) percent in both seasons, so this GA_3 level the maximum seed yield (1892.7 and 1931.25 Kg) in both seasons, respectively compared with the other GA3 treatments and control (1618.25 and 1716.75). in both seasons, respectively. Therefore, application of GA_3 150 ppm is recommended as it achieved the maximum improvement in the vegetative and reproductive growth which leaded to raise the economic value of the jojoba plant as a promising tree for planting in drought, salinity area to get its seed commercial oil which potentially useful as a biofuel with multi chemical and pharmaceuticals industries. The maximum seed lipids content (55.13 and 55.94%) were produced in 150ppm GA3 treatment in both seasons, respectively compared with the other GA_3 treatments and control, this Egyptian jojoba seed oil contained C20:1 is the major constituent followed by C18:1, C22:1 and 24:1 respectively, whereas C14:1 was present at low concentration amounted.*

Key Words: *Jojoba, gibberellic acid (GA_3), vegetative growth, flowering, fruit set, seed yield and seed oil fatty acid content.*

Date of Submission: 15-02-2018 Date of acceptance: 01-03-2018

I. Introduction

Jojoba (*Simmondsia chinensis*) belongs to the family Simmondsiaceae.) precious, drought resistant shrub which mostly a woody, evergreen dioecious, perennial shrub that produces small brownish seeds which have a high liquid wax content, is considered a promising oil crop and is cultivated for diverse purposes in many countries for more than 30 years in many desert and semi-desert areas worldwide, such as India, Mexico, Chile, Australia, Saudi Arabia and Egypt, so it is being endemic to the Sonoran Desert in southern Arizona, southern California and northern Mexico (Abd-El-Mageed et all, 2014). The jojoba seed produces unique high-quality oil with a wide range of applications such as medical and industrial-related products.

Jojoba seed are brown, elongated, slightly spherical and wrinkled in shape, its contain 50-60% of light yellow, odorless liquid wax ester which call jojoba oil. (Mohamed et all 2017). The Egyptian seeds are rich in wax esters (55 %) with fatty alcohols C20:1 and C22:1 as major components and amounted to 43.0 % and 45.6 % respectively (El-Mallah and El-Shami , 2009)

Jojoba seed oil or wax is used as a natural base for a wide range of cosmetic products for skin, hair and face because of its safety, purity, lack of odor and stability. In addition, it also possesses heat resistant lubricating properties and is potentially useful in the chemical industry as a basic feedstock such as pharmaceuticals, lubricants, gear additives, extenders, anti-foaming agents, and in the wax and polish industries (US National Research Council, 1985 and Wisniak, 1987). Many studies mentioned the antifeedant, antifungal, insecticidal actions of jojoba (Al-Obaidi et al.,2017). In addition to the plant extracts induced variable degrees of antioxidant activity and proposes its potential as an antioxidant agent for pharmaceutical uses (Al-Qizwini et al.,2014.)

There are a lot of factors control vegetative growth, flowering, fruiting of jojoba plant and their seed yield and it's chemical consistent such as plant growth regulators. Gibberellic acid GA_3 is a natural growth hormone and is a part of a type of plant hormones called gibberellins. GA_3 promotes cell division and many plant development mechanisms and encourages numerous desirable effects such as plant height, uniform flowering, reduced time to flowering and increased flower number and size. It has been well documented that the size and quality of the fruits can be affected with the application of plant growth hormones (Guardiola, 1992). Gibberellic acid has been shown to increase fruit set and growth in clementine orange (Van Rensburg et al., 1996). It is well documented in the literature that gibberellic acid is used widely in horticultural crops for improving fruit set (Taylor and Knight 1986). Spray Gibberellins are known for their ability to increase cell enlargement (Davis, 2004; Pharis and King, 1995), thus enhancing fruit growth in certain species such as citrus (Eman et al., 2007), guava (El-Sharkawy et al., 2005), and pear (Zhang et al., 2007). In all species so far studied, gibberellins had the potential for increasing fruit size. The beneficial effects of gibberellic acid (GA_3) on yield and fruit quality of different fruit crops were mentioned by many investigators including Swietlik (2002). The results showed that the *Schefflera arboricola* plants sprayed with gibberellic acid at 300 ppm gave the highest values of plant height, number of leaves, stem diameter, fresh and dry weight of leaves and stems and dry weight of roots, however, leaf area and fresh weight of roots increased with application of 200 ppm. In addition, chlorophyll a, b and carotenoid as well as total carbohydrates and nutrients (nitrogen, phosphorus and potassium) increased with 300 ppm (Azza et all, 2014)

Since no studies has been done about the effect of gibberellic acid on plant vegetative growth, flowering, fruit set , seed yield and the traits of seed oil content in jojoba plants under Egyptian semi-arid soil conditions, so the aim of this work was to study and the possibility improvement of these traits as well as seed oil yield in jojoba plants by using gibberellic acid foliar application.

II. Materials And Methods

This experiment was conducted to find out the effect of foliar application of gibberellic acid on Jojoba (*Simmondsia chinensis*), at privet farm in EL-Behira governorate, Egypt. Jojoba trees of similar vigor, age (nine years old) and size were selected for sprays treatments during 2015 and 2016 season. The experiment laid out is arranged in Randomized Complete Block Design (RCBD) with three replications. For each replication of treatment, same shoot with regard to height, thickness, vigor and number of fruit and orientation was selected. Distances between rows and within plants in rows were 3 and 2.5 m, respectively. Drip irrigation system was applied in the orchard, weed and pest control, and fertilization conducted following the standard agro-management practices. All the studied plants were subjected to the same condition including irrigation, farm practices and…. etc. The orchard soil analysis is given in (Table 1) according to procedures (Chapman,1961).

Table (1): Some physical and chemical analysis of the experiment soil

parameters	pH	EC(dSm-1)	Ca++	Mg++	Na+	K+	HCO3-	Cl-	SO4=
values	7.88	2.26	2.14	3.78	19.66	0.31	1.11	21.25	4.56

Parameters were recorded to achieve the objectives of this experiment for measuring the response of plant vegetative growth and reproductive characters of jojoba to the application of spraying gibberellic acid. These parameters were measured as follows: For each treatment nine plants (three plants for every replicate) by randomly chosen were tagged. Data were collected from tagged plants of each treatment for each replicate. GA_3 were applied three times (in the beginning of December, March and May). The gibberellic acid (GA_3) treatment doses were 0, 50, 100 and 150 ppm. Treatments were performed by spraying 4 liters per shoots of the GA_3 solutions in the first and second seasons, respectively

1: Vegetative characters: Three branches were selected from the mid- level of each plant and the average of their characters were calculated for detailed analysis. On each branch the following data were collected:

1.1: branch length (cm)
1.2: secondary branches length(cm)
1.3: Number of branches nodes.

2: Reproductive characters: Three Branches per plant from each treatment were tagged in December 2014and2015, and the number of floral buds was recorded. Every 15 days the number of open buds (with a visible stigma) was recorded, and the flowering percentage was calculated.

2.1: Flowering date: The numbers of days from first January until open the flowers was recorded. When 50% of the flowers were opened, the flowering date was calculated and recorded.

2.2: Initial Fruit set percentage: Four weeks after full bloom initial fruit set percentage on replicate trees of the studied treatments was calculated from the following equation according to Yehia and Hassan (2005) as the following equation:

Initial fruit set (%) = Number of fruit/m x 100/ Average number of flowers/m

2.3: Final Fruit set %: The ratio of the number of fruitlet to the number of total flowers branch at the end of March

Percentage of fruit set = (No. of fruitlets x 100)/ Total No. of flowers.

2.4: Seed yield: Seed were harvested from the previous tagged plants by hand in the two seasons at full maturity. Harvested seeds were cleaned, dried and weighted (g) to calculate the total seed yields per Fed (4100 m^2).

3: Seed Chemical traits

Chemical analyses were performed following the AOAC (1995)

3.1: lipid content: To determine oil content, seeds of each treatment were randomly selected, weighed, and dried at 50 ºC. The drying process was continued until the difference between the two successive weights was less than 1 mg. three replications were used for this characteristic. The oil was extracted for 16 h with hexane with a Soxhlet apparatus.

3.2: Crude protein: Total organic nitrogen (N) was determined according to the method of Kjeldahl as indicated by (AOAC, 1995) for dry material. Crude protein content was obtained by multiplying the nitrogen (N) value by 6.25. Data represent the means of five replications.

3.3: Total carbohydrates: Total carbohydrates were estimated by the difference in the mean values, i.e., 100 - (sum of concentrations of protein, ash and lipid).

3.4: Seed oil fatty acid composition:

The fatty acid composition of the seed oil was determined by conversion of oil to fatty acid methyl esters prepared by adding 950 µl of n-hexane 50 mg of oil followed by 50 µl of sodium methoxide using the method of Cocks et al., (1966). The mixtures were vortex for 5 s and allowed to settle for 5 min. The top layer (1 µl) was injected into a gas chromatograph (Model GC- 14A , Shimadzu Corporation, Kyoto, Japan) equipped with a flame-ionisation detector and a polar capillary column (BPX70 0.25), 0.32 mm internal diameter, 60 m length and 0.25 :m film thickness (S GE Incorporated , US A) to obtain individual peaks of fatty acid methyl esters. The detector temperature was 240 °C and column temperature was 110 °C held for one minute and increased at the rate of 8 °C.min^{-1} to 220 °C and held for one minute. The run time was 32 min. The fatty acid methyl esters peaks were identified by comparing their retention time with those of standards. Percent relative fatty acid was calculated based on the peak area of a fatty acid species to the total peak area of all the fatty acids in the oil sample.

5: Statistical Analysis

The experiment was conducted in factorial lay out arranged in completely randomized block design with three replications. Analysis of variance with SAS software (SAS Institute, 1988) was carried out on the test clone's data. Clones' means were compared using the LSD test at 5% level of probability.

III. Results And Discussion.

3.1: Vegetative parameters

Vegetative parameters in table (2) indicate that foliar application of GA_3 promoted the branch length and secondary branches length, compared to the corresponding untreated control plants during two studied seasons, respectively. Moreover, these branches and secondary branches length parameters significantly increased with increasing the levels of GA_3 in both seasons. The treatment of 150ppm GA_3 recorded the highest means of branch length (95.09 and 98.68) and secondary branches length (50.29 and 52.84) in both seasons, respectively. From the other hand, the control treatment (0ppm GA3) recorded the lowest values of branch length (66.22 and 73.58 cm) and secondary branches length (22.88 and 24.55 cm). Regarding the minimum number of branched nodes (2.68 and 2.47) was found in the treatment 150ppm GA_3 in the first and second seasons, respectively (Table2). Moreover, the results of GA_3 agree with those obtained by Abou-Sedra (1981) who reported that GA3 application resulted in an increase in plant height, number of leaves and fresh and dry

weight in spinach plants. Wanyama *et al*. (2006) found that the application of GA3 increased branching of Cape gooseberry plants.

Table (2): The mean of branch length (cm), secondary branches length (cm) and number of branched nodes of jojoba plants as affected with GA_3 levels.

Treatment	**Branch length (cm)**		**Secondary branches length (cm)**		**Branched nodes**	
	first	**second**	**first**	**second**	**first**	**second**
0 ppm Ga	72.12 d	75.92 d	32.99 d	35.15 d	3.857a	3.48a
50 ppm Ga	78.08 c	81.22 c	38.76 c	42.67 c	3.327ab	3.02ab
100 ppm Ga	87.50 b	91.73 b	42.59 b	46.70 b	2.88b	2.695ab
150 ppm Ga	95.09 a	98.68 a	50.29 a	52.84 a	2.685b	2.477b
L.S.D.	0.8952	1.1102	0.5678	0.6734	0.7384	0.7858

3.2: Flowering date, initial fruit set, final fruit set and seeds yield:

Spraying GA_3 table (3) at any concentration significantly increased the initial fruit set and final fruit set percentage but decreased the number of days of fifty percentage flowering comparing with the control. Results showed in table that the maximum final fruit set (91.05 and 91.23) percent were produced in 150ppm GA3 treatment in both seasons, so this GA_3 level gave the minimum 50% flowering (41.24 and 37.50) days respectively compared with the other GA_3 treatments and control. The obtained results of the effect of GA_3 application on jojoba are in line with many reports such as, Gibberellic acids (GA_3) have been shown to increase fruit set, growth, yield and fruit weight of grapevines (Zabadal and Dittmer 2000). Improving le-Cont pear trees productivity by spraying GA3 (Mostafa et al. 2001). Stern and Gazit (2003) also hypothesize that sprays of GA3 during stage I of fruit growth would increase fruit and aril weight. Balady mandarin trees sprayed with GA3 resulted in increased number of fruits/ tree (El-Sese 2005). GA3 significantly increased the total number of fruits, the fruit weight per plant by reducing pre-harvest fruit drop in orange (Davies and Zalman 2006). The application of GA3 increased flower bud formation and fruiting of Cape gooseberry plants (Wanyama *et al*., 2006). Mohammad et all. (2015) found that 50 mg L-1 GA3 once each week from the beginning of flower opening through fruit development, exhibited highest chlorophyll fluorescence and quantum yield, stimulated PAL (Phenylalanine ammonia-lyase) activity and anthocyanin biosynthesis of the jambu air madu fruits under field conditions. In seeds yield, results in table (3) showed that the GA_3 treatment significantly increased seed yield, so the maximum seed yield (1892.7 and 1931.25 Kg) were produced in 150ppm GA_3 treatment in both seasons, respectively compared with the other GA3 treatments and control (1618.25 and 1716.75). The increasing in seeds yield due to GA_3 sprays may be attributed to their effects on increasing levels of IAA. The role of GA_3 in improving fruit quantity which reflected on seed yield may be due to its role in increasing cell elongation (Eman et al., 2007). Marschner (1986) indicated that application of GA3 and/or IAA on higher plants caused elongation in the primary cells in the young tissues and growth centers. The present results may be attributed to simulative influence of this bioregulator on cell extension and /or on cell division. GAs increase sink demand by the enhancement of phloem unloading or/and metabolism of carbon assimilates in fruit. The GA_3 delays senescence, improves growth and development of chloroplasts, and intensifies photosynthetic efficiency which could lead to increased yield (Yuan & Xu 2001, El-sese , 2005).

Table 3: The mean of flowering date, initial fruit set %, final fruit set % and seed yield as affected with gibberellic acid

Treatment	**Flowering date (day)**		**Initial fruit set%**		**final fruit set%**		**Seeds yield (kg)/Feed***	
	First season	**second season**	first **season**	second **season**	**first season**	**second season**	**First season**	**Second season**
0 ppm GA	55.75 a	52.25a	90.68 d	91.49 c	89.03 d	89.55 c	1618.25d	1716.75d
50 ppm GA	49.25 b	46.00 b	91.66 c	92.42 b	90.06 c	90.36 b	1707.75b	1716.75c
100ppm GA	45.50 c	42.50 c	92.12 b	92.45 b	90.48 b	90.94 b	1803.00b	1844.75a
150ppm GA	41.25 d	37.50 d	92.54 a	92.96 a	91.05 a	91.23 a	1892.75a	1931.25a
L.S.D.	1.0422	1.0226	0.1572	0.1971	0.3151	0.2807	50.291	45.105

***Feed: 4100 m^2**

3.3: Seed chemical traits:

Results in table (4) showed that the maximum seed lipids content (55.13 and 55.94%) and minimum seed content of protein (23.99 and23.77%) were produced in 150ppm GA3 treatment in both seasons, respectively compared with the other GA_3 treatments and control. In addition, the highest content of the carbohydrate (19.50 and 19.17%) was found with using 150ppmGA_3 in the first season and second seasons, respectively. Abd El-Migeed (2002) found that improving productivity and fruit quality of Washington navel orange trees by using some macro-elements and GA_3 sprays. El- Sharkawy and Mehaisen (2005) observed that gibberellin foliage sprays enhance productivity and fruit quality of guava trees. Kuiper (1993) suggested that sink strength is established and regulated by plant growth regulators. That is, certain plant hormones can increase mobilization of assimilates to fruit and modulate many of the rate-limiting components in carbon partitioning (Ozga and Dennis, 2003). A larger fruit which contain seeds and increased sink demand were closely correlated with changes in activities of sugar metabolizing enzymes induced by GA_3 application. Hagagg et al., (2014) found that the combination between zinc sulfate and gibberellic acid increased fruit yield and oil content of olive trees cv. Kallamata.

Table (4). The mean of seed lipids, proteins and carbohydrates as affected with gibberellic acid levels.

Treatment	**Lipids%**		**Proteins%**		**Carbohydrates%**	
	first season	**second season**	**First season**	**second season**	first **season**	second **season**
0ppm GA	52.07 d	52.68 d	27.34 a	26.69 a	19.06 d	19.07ab
50ppm GA	53.22 c	53.67 c	26.04 b	25.65 b	19.28 c	18.87bc
100ppmGA	54.29 b	55.01 b	24.94 c	24.86 c	19.42 b	18.68 c
150ppmGA	55.13 a	55.94 a	23.99 d	23.77 d	19.50 a	19.17a
L.S.D.	0.3883	0.2252	0.421	0.4372	0.0446	0.2263

Seed Oil fatty acid composition:

Jojoba Oil is a liquid wax ester and not an oil. It is a natural emollient with good spread ability to be used to provide a protective coating on the skin. It is also useful for hair conditioning agent.

The results were shown in Table (5) the fatty acids content of Jojoba seed oil, as in the results, based on GC analyses, of the Jojoba oil indicated that the oil contains fatty acids of carbon atoms from C 14 to C 24 (saturated and unsaturated).The main fatty acids constituents (average) of Jojoba seed wax are ecosenoic acid (56.05) oleic acid (15.35) and erucic acid (12.75) and legnoceric acid (10.67)

The fatty acids profile showed that C20:1 is the major constituent followed by C18:1 and C22:1 and 24:1 respectively) whereas C14:1 was present at low concentration amounted. In addition, the Egyptian jojoba wax contained C18:2 fatty acid at a level of 1.9 %.These results was agreed with El-Mallah and El-Shami (2009) in three fatty acids contain who found that C20:1 is the major constituent (60 %) followed by C18:1 and C22:1 (14.5 and 11.8 % respectively), but not agreed with them about other fatty acids results whereas C18:2 fatty acid at a level of 8.7 % and C24:1 was present at low concentration amounted to 1.6 %.

Table (5): Gas chromatography (GC) for jojoba seed oil fatty acids

Fatty acids	1	2	3	4	5	6	7	8
C14:0 Myristic acid	0.49	0	2.56	3.36	3,49	2.06	1.02	2.16
C14:1 Mrisoleic acid	0	0	0	2.43	0	1.17	0	0
C16:0 Palmitic acid	1.85	1.64	1.96	1.69	1.75	1.22	1.43	1.71
C18:1 Oleic acid	17.15	16.78	16.88	15.31	16.89	15.82	15.36	14.82
C18:2 linoleic acid	1.7	1.9	2	1.9	1.8	2.1	2	1.8
C20:1Ecosenoic acid	57.33	57.87	54.49	53.41	51.07	58.22	57.41	60.43
C22:1 Erucic acid	12.24	13.81	13.75	12.38	13.61	11.04	12.42	11.67
C24:1Lignoceric acid	10.94	10.09	10.36	10.97	12.74	10.47	12.36	9.21

IV. Conclusion

By comparing means of treatments, it was concluded that there were significant effects of GA_3 treatments on all jojoba studied parameters. Moreover, jojoba vegetative and reproductive parameters and seed oil content enhanced with increasing the level of GA_3. So, the treatments of 150ppm GA_3 increased the yield scientifically and gave the best results in all studied traits in both seasons compared with the other gibberellic acid treatments and control. The Egyptian jojoba seed oil contained C18:2 fatty acid at a level of 1.9 %, in addition to the fatty acids profile showed that C20:1 is the major constituent followed by C18:1, C22:1 and 24:1 respectively) whereas C14:1 was present at low concentration amounted.

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*Dr.Sudad K.AL-Taweel. " Oil Content, Vegetative And Reproductive Traits of Jojoba Plant As Affected By Foliar Application of Gibberellic Acid ." IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 11.0 (2018): 53-58.