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A NEW ENVIRONMENTAL FRIENDLY PRODUCTION METHOD IN SUNFLOWER FOR HIGH SEED AND CRUDE OIL YIELDS

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ABSTRACT

Biotic (pests, diseases and weeds) and abiotic (drought, cold, heat and salinity) stress factors cause to yield loss. Improvement of resistant or tolerant cultivar against these stress factors is the main target of plant breeding. Pesticides and other chemicals have been widely used to obtain high yield by fighting biotic stress factors (pests, diseases and weeds). However, use of chemicals in agriculture causes extra financial cost and environmental pollution. That is why, improvement of new methods for high yield is obligatory. Leaves in plants form active surface of photosynthesis. High photosynthetic activity affects yield directly by increasing matter production. The aim of this study was to increase photosynthetic activity in sunflower via leaf-removal (defoliation) and consequently to increase seed, crude protein and crude oil yields by increasing photosynthetic activity. Oil type sunflower cultivars used in the study ('08-TR-003', 'TR-3080' and 'TARSAN-1018') were obtained from "Trakya Agricultural Research Institute". When plants reached to star-shaped head stage which is the beginning of generative stage, 4 different leaf-removal treatments which were control (no leaf-removal), 2 leaves-removal, 4 leaves-removal and 6 leaves-removal, were performed. Half of the leaves was removed from just below the head while other half was removed from the middle part of the plant in each treatment. After harvest, seed yield per plant, seed yield per decare, crude protein percentage, crude oil percentage, crude protein yield per decare and crude oil yield per decare were calculated. At the end of the study, it was observed that a certain number of leaf-removal for each cultivar compared to control affected all agronomic characters positively.

KEYWORDS:
Sunflower, leaf removal, seed yield, crude protein yield, crude oil yield

INTRODUCTION

Plantal production should be increased in order to meet the food demand of increasing world population. This can only be achieved by increasing yield since sowing areas cannot be enlarged. Furthermore, cultivated areas which cover 3% of the total world surface, are getting narrowed rapidly due to erosion, salinity, acidity, unplanned urbanization, intensive agriculture and extreme grazing. It is estimated that world population will be 11 billion in 2050 [1]. With the effects of all these factors and increasing population, it is estimated that cultivated area per capita will be 0.15 hectare in 2050. On the other hand, food production is negatively affected by the changes of world climate caused by global warming. More than 30% of crop production are lost due to biotic (pests, diseases and weeds) and abiotic (drought, cold, heat and salinity) stress factors. Improving new cultivars resistant to stress factors is the main target of plant breeding. Fighting against biotic stress factors which decrease crop production, is achieved by chemical methods. However, careless usage of chemicals such as fertilizers, pesticides and herbicides disturbs ecological balance. Improving resistant cultivars by conventional and modern techniques against biotic and abiotic stress factors is very difficult since resistance is occurred by additive gene effect. That is why, new environmental friendly production methods should be improved to increase yield per unit area against rapidly increasing world population and decreasing natural sources.

Leaves form the active photosynthetic area in plants [2, 3]. High photosynthetic activity increases yield. In greenhouse studies conducted by us showed that leaf-removal (defoliation) till a certain number increased photosynthetic activity. "Star-Shaped Head Stage" in sunflower is the beginning of reproductive period. Substances formed by photosynthesis are stored in seeds in this period. Higher photosynthetic activity in this period increases the main agronomic characters such as seed, crude protein and crude oil yields. Preliminary studies conducted in different species by us showed that leaf-removal at a certain number gave rise to increased photosynthetic activity.

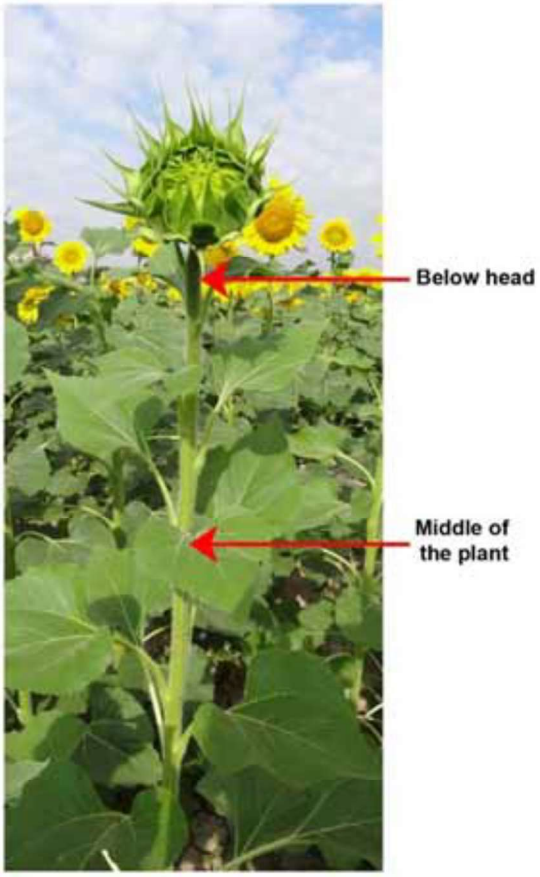

FIGURE 1
The places where leaves were removed in "Star-Shaped Head Stage"

The aims of this study are to increase seed, crude protein and crude oil yields by increasing photosynthetic activity via leaf-removal from the plant in "Star-Shaped Head Stage" which is the beginning of reproductive period.

MATERIALS AND METHOD

This study was conducted in the years of 2013 and 2014 in experimental fields of Faculty of Agriculture, Ankara University.

Plant Material. Oil type sunflower cultivars '08-TR-003', 'TR-3080' and 'TARSAN-1018' obtained from "Trakya Agricultural Research Institute" were used in the study.

Soil Preparation, Planting and Plant Development. Soil of trial field was ploughed 30 cm in depth in fall before winter. In spring, it was ploughed again 10-15 cm in depth to make soil ready for planting. Planting was performed in the first week of April with spaces of 70 cm row width and 25 cm on-row. Three seeds were put in every hole to guarantee the emergence. Two weeks after emergence, two of the plants were eliminated and only one plant left in every hole.

For all leaf-removal treatments in all cultivars, plots were fertilized with 14 kg/da diamoniumphosphate (DAP) before planting. During growing, weed control was achieved by hand in experimental field. Plants were irrigated during development according to water need of the plants. After flowering and fertilization, heads of the plants from which observations would be performed, were covered by paper bags to avoid birds' harm. Plants were harvested when 80% of sunflower heads were brown.

Leaf-Removal (Defoliation) Treatments. When plants reached to "Star-Shaped Head Stage" which is the beginning of reproductive period, 4 different leaf-removal treatments were performed. These are: control (no leaf removed), 2 leaves-removal, 4 leaves-removal and 6 leaves-removal. Half of the leaves was removed from just below the head while other half was removed from the middle part of the plant for each leaf-removal treatment as shown in (Figure 1).

Determination of Total Chlorophyll Content. Total chlorophyll content was determined in leaves of plants according to the protocol of Curtis and Shetty [4]. Fresh tissue of 50 mg from leaf was put in 3 ml methanol and kept in total darkness at 23ºC for 2 hours. By this way, chlorophyll in fresh tissue passed through into methanol. After 2 hours, absorbencies were determined at 665 and 650 nm. Total chlorophyll content was calculated as "µg chlorophyll/g fresh tissue".

Lipid Peroxidation. Lipid peroxidation was determined by measuring the amount of MDA produced by the thiobarbituric acid reaction as described by Lutts [5]. A 200-mg sample of fresh leaves were squashed with a 5-mL portion of a 0.1% tricholoroacetic acid (TCA) solution, and the mixture was centrifuged for 20 min at 12 500 rpm. A 3-mL portion of the supernatant was then taken out of the 5-mL extract. After that, a 3-mL portion of a solution containing 20% of tiobarbituric acid and 20% of tricholoroacetic acid was added to 3 mL of the supernatant. The absorbencies of supernatant monitored at 532 and 600 nm with the spectrophotometer.

Antioxidant Enzyme Activity. For enzyme assays, fresh leaf samples (1 gram) were ground to a fine powder with liquid nitrogen in a ceramic mortar and homogenized with 10-mL portions of a 50 mM phosphate buffer solution containing 0.1 mM of Na-EDTA. Homogenized samples were centrifuged at 15 000 rpm for 15 min and then used for enzyme analysis.

The activity of superoxide dismutase (SOD) was measured using the method of Cakmak and Marschner [6] and Cakmak [7]. According to this method, a 3 mL of a 50 mM phosphate buffer (pH 7.6) containing 0.1 mM of Na-EDTA; the enzyme extract (0.025–0.1 mL); 0.5 mL of a 50 mM solution of Na_2CO_3 (pH 10.2); 0.5 mL of a 12 mM solution of L-methionine; 0.5 mL of a 75 µM solution of nitro blue tetrazolium chloride (NBT); and, finally, 0.01 mL of riboflavin were added into glass bottles to prepare solutions. The absorbencies of the solutions were read at 560 nm 15 min after the mixing.

Catalase (CAT) activity was determined according to Cakmak and Marschner [6]; Çakmak [7] who measured the rate of the decrease of absorbance of H_2O_2 at 240 nm. The reaction mixture contained 0.8 mL of a 50-mM phosphate buffer solution (pH 7.6) containing 0.1 mM of Na-EDTA, 0.1 mL of a 100 mM H_2O_2 solution and 0.1 mL of the enzyme extract. The volume of the reaction medium was adjusted to 1 mL. The absorbencies of the resulted solutions were monitored at 240 nm.

The activity of ascorbate peroxidase (APX) was assayed according to method described by Cakmak and Marschner [6] and Cakmak [7]. The mixture containing a portion of a 50-mM phosphate buffer solution (0.7 mL, pH 7.6, also containing 0.1 mM of Na-EDTA) was mixed with 0.1 mL of a 12 mM solution of H_2O_2, also containing 10 mM EDTA, 0.1 mL of a 0.25 mM solution of L(-)-ascorbic acid and 0.1 mL of the enzyme extract. The total volume of the mixture was adjusted to 1 mL, and its absorbance was read at 290 nm.

The activity of glutathione reductase was determined according to protocols by Cakmak and Marschner [6] and Cakmak [7]. According to these protocols, 1 mL of the assay mixture was prepared like this: a 0.7-mL portion of a 50 mM phosphate buffer solution (pH 7.6, also containing 0.1 mM of Na-EDTA) was mixed with 0.1 mL of a 0.5 mM oxide glutathione (GSSG) solution, 0.1 mL of a 0.12 mM NADPH solution and 0.1 mL of the enzyme extract. Absorbance of the mixture was measured at 340 nm.

Observations. Observations were performed in totally 30 plants (10 plants per replication) in each leaf-removal treatment of all cultivars. At the end of the study, seed yield per decare (kg da^{-1}), crude protein yield (kg da^{-1}) and crude oil yield (kg da^{-1}) were determined as agronomic characters while total photosynthetic activity, lipid peroxidation (MDA), superoxide dismutase (SOD), activities of catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) were recorded as physiologic characters.

Statistical Analysis. Experiments were arranged at 'Randomized Complete Block, Split-Plots' design with 3 replications. In the experiment, oil type sunflower cultivars were main plots and 4 leaf-removal treatments were sub-plots. Data were statistically analyzed by Duncan's multiple range test using 'SPSS for Windows'. Data given in percentages were subjected to arcsine ($\sqrt{X}$) transformation before statistical analysis [8].

RESULTS AND DISCUSSION

From one hand, current food production is insufficient against rapidly increasing world population which is estimated to be 11 billion in 2050, on the other hand cultivated areas covering 3% of the total world surface area are getting narrowed rapidly due to erosion, salinity, acidity, intensive agriculture and extreme grazing. It is estimated that cultivated area per capita will be 0.15 hectare in 2050. In addition, finding required water sources for modern agriculture will be more difficult because of increasing water consumption and water dirtiness. More than 30% of our crop yields are lost due to biotic and abiotic stress factors. That is why, high-yielded new cultivars should be improved to meet food demand of increasing world population. However, improvement of new cultivars resistant to biotic and abiotic stress factors is extremely difficulty by breeding methods since characters controlling resistance to stress factors are determined by additive genes effect. And also improving new cultivars takes more time, needs working in wide areas and labor expenses increase the cost.

There are research studies examining the effects of leaf-removal on seed and crude oil yields in sunflower [9, 10, 11, 12, 13]. However, in all these studies, it was reported that leaf-removal from plant gave rise to decreases in seed and crude oil yields. It was thought that the reason of these negative results could be due to use of improper methods. Thus, in some of the studies, all leaves in plant were removed while 1/3 or 2/3 of leaves were removed in some studies. Or leaves from the bottom of the plant were removed. Whereas, it was reported that effective leaves on yield were in top and middle of the plant [14]. In our study, leaves at a certain number from top (below head) and middle of the plant were removed to modify the yields of seed and crude oil.

The effect of different leaf-removal treatments on seed, crude protein and crude oil yields were shown in (Table 1.) From the results, it could be concluded that leaf-removal from the plant at a certain number affected seed, crude protein and crude oil yields significantly. The highest results in all the parameters were recorded from leaf-removal treatments compared to control in which no leaf was removed.

Seed yield was recorded as 385.4 kg da^{-1} in control while it was 431.2 kg da^{-1} in the treatment of 4-leaf-removal as the highest in cv. '08-TR-003'. In

cv. 'TR-3080', seed yield increased to 432.7 kg da^{-1} in 2-leaf-removal treatment from 398.3 kg da^{-1} in control. Seed yield was realized 407.3 kg da^{-1} in control treatment while it was 451.6 kg da^{-1} in 6-leaf-removal treatment in cv. 'TARSAN-1018'. Increase percentage in seed yield compared to control was recorded as 11.87% in cv. '08-TR-003', 8.64% in cv. 'TR-3080' and 10.87% in cv. 'TARSAN-1018'. Mean of increase percentage of three cultivars in seed yield was calculated as 10.46% (Table 1).

The highest results in crude protein yield were recorded from the treatments of 4-leaf-removal, 2-leaf-removal and 6-leaf-removal in cvs. '08-TR-003', 'TR-3080' and 'TARSAN-1018', respectively. Increase percentage compared to control was noted 1.90%, 6.40% and 12.61% in cvs. '08-TR-003', 'TR-3080' and 'TARSAN-1018', respectively. Mean of increase percentage of three cultivars in crude protein yield was 6.97% (Table 1).

Crude oil yield increased to 207.7 kg da^{-1} in 4-leaf-removal treatment from 175.0 kg da^{-1} in control in cv. '08-TR-003'. In cv. 'TR-3080', crude oil yield was 184.8 kg da^{-1} in control treatment while it was 209.5 kg da^{-1} in 2-leaf-removal treatment in cv. 'TR-3080'. In cv. 'TARSAN-1018', the highest value in crude oil yield was noted as 215.3 kg da^{-1} in 6-leaf-removal treatment while it was 190.7 kg da^{-1} in control. Increase percentage in crude oil yield compared to control was obtained as 18.67%, 13.36% and 12.92% in cvs. '08-TR-003', 'TR-3080' and 'TARSAN-1018', respectively. Mean of increase percentage of three cultivars in crude oil yield was 14.98% (Table 1).

In the current study, the highest values in total chlorophyll content were recorded in 4-leaf-removal, 2-leaf-removal and 6-leaf-removal treatments in cvs. '08-TR-003', 'TR-3080' and 'TARSAN-1018', respectively. In cv. '08-TR-003', total chlorophyll content was noted as 1235.30 µg/g fresh tissue while it was 1023.28 µg/g fresh tissue in control. Increase percentage according to control was realized as 33.42%. Total chlorophyll content was recorded as 1473.44 µg/g fresh tissue while it was 1011.01 µg/g fresh tissue in control treatment in cv. 'TR-3080'. Increase percentage according to control in total chlorophyll content was noted as 45.74%. In cv. 'TARSAN-1018', total chlorophyll content was 1463.75 µg/g fresh tissue in 6-leaf-removal treatment as the highest while it was 1100.33 µg/g fresh tissue in control treatment in which no leaf was removed. And increase percentage according to control was 33.02% in cv. 'TARSAN-1018'. These results confirmed that leaf-removal from plant significantly increased chlorophyll content which cause to higher photosynthetic activity and yield. In our study, the highest total chlorophyll contents in all cultivars were recorded from leaf-removal treatments from which the highest seed, crude protein and crude oil yields were obtained. Highest results in seed, crude protein and crude oil yields could be attributed to highest total chlorophyll contents which caused to higher photosynthetic activity. It was reported that there is a close relationship between photosynthesis and chlorophyll content [15]. Chlorophyll content of leaf is accepted as an indicator of photosynthetic capacity of tissues [16-18] and the amount of it in tissue is changed under stress conditions [19-21].

The mean values of antioxidant enzyme (SOD, GR, APX and CAT) activities and lipid peroxidation (MDA) contents in leaf-removal treatments in sunflower plants are shown in Table 2. It was observed that in all physiologic characters, the highest results were obtained from leaf-removal treatments compared to control.

TABLE 1
The effect of leaf-removal treatment on seed, crude protein and crude oil yields in sunflower

Cultivars	Number of Leaves Removed from Plant	Seed Yield (kg da^{-1})	Increase Percentage in Seed Yield According to Control (%)	Crude Protein Yield (kg da^{-1})	Increase Percentage in Crude Protein Yield According to Control (%)	Crude Oil Yield (kg da^{-1})	Increase Percentage in Crude Oil Yield According to Control (%)
'08 -TR- 003'	0 (Control)	385.4±3.7 c	11.87	70.4±1.1 a	1.90	175.0±4.2 c	18.67
	2	371.9±4.7 d		62.8±1.1 b		173.1±1.1 c	
	4	431.2±1.5 a		71.7±1.3 a		207.7±1.2 a	
	6	399.0±4.0 b		68.2±0.5 ab		185.1±1.8 b	
'TR - 3080'	0 (Control)	398.3±0.6 ab	8.64	66.2±0.8 b	6.40	184.8±1.5 b	13.36
	2	432.7±4.5 a		70.4±0.7 a		209.5±1.6 a	
	4	388.1±3.8 b		67.7±0.8 b		184.7±2.1 b	
	6	376.6±5.5 b		59.2±1.1 c		173.8±1.3 c	
'TARSAN - 1018'	0 (Control)	407.3±4.4 b	10.87	75.6±0.9 c	12.61	190.7±1.3 c	12.92
	2	425.3±2.3 ab		77.1±0.9 c		199.7±1.7 bc	
	4	443.4±1.3 a		81.0±1.0 b		206.0±6.0 b	
	6	451.6±1.2 a		85.1±0.8 a		215.3±1.5 a	
Overall Mean			**10.46**		**6.97**		**14.98**

Values within a column followed by different letters are significantly different at the 0.01 level for each cultivar
All values is the mean of years 2013 and 2014

TABLE 2
The effect of leaf-removal treatment on physiologic characters in sunflower

Cultivars	Number of Leaves Removed from Plant	Total Chlorophyll Content (µg/g fresh tissu)	Increase Percentage According to Control (%)	Lipid Perox. (MDA) (µmol/g fresh weight)	Increase Percentage According to Control (%)	Superoxide Dismut. (SOD) (unit/min./mg fresh weight)	Increase Percentage According to Control (%)	Catalaz (CAT) Activity (unit/min./mg fresh weight)	Increase Percentage According to Control (%)	Acrorbate Perx. (APX) Activity (unit/min./mg fresh weight)	Increase Percentage According to Control (%)	Glutation Reductase (GR) Activity (unit/min./mg fresh weight)	Increase Percentage According to Control (%)
'08 -TR- 003'	0 (Control)	1023.28	33.42	19.9±0.2 c	26.86	168.8±12.4 c	35.21	867.6± 30.6 b	21.94	2314.6±151.6 b	17.93	52.2±3.5 ab	18.82
	2	1235.30		25.2±0.6 a		193.2±3.4 b		1058.1±37.1 a		1616.1±4.3 c		49.5±4.1 b	
	4	1365.32		18.8±0.4 c		224.5±6.9 a		758.7± 22.8 b		2729.7±65.2 a		61.8±1.7 a	
	6	1119.16		23.0±0.3 b		228.3±3.3 a		752.7±69.8 b		2569.3±3.4 ab		62.0±3.3 a	
'TR - 3080'	0 (Control)	1011.01	45.74	23.8±0.3 c	35.14	162.5±2.2 c	24.74	828.6± 12.5 ab	5.19	2366.1±164.5 b	27.11	62.2±5.7 a	5.08
	2	1473.44		23.0±0.2 c		179.4±0.3 b		591.8± 16.1 c		3007.6±196.4 a		65.4±17.0 a	
	4	1211.76		32.1±0.3 a		193.9±2.5 a		871.6± 52.6 a		2403.6±206.6 b		58.3±3.3 b	
	6	1033.82		28.2±0.8 b		202.7±6.6 a		749.3±10.5 b		1945.4±63.7 b		46.9±2.2 b	
'TARSAN - 1018'	0 (Control)	1100.33	33.02	19.9±0.3 b	18.30	212.8±10.1 b	35.28	751.6± 49.4 b	28.98	2107.9±44.7 b	37.83	61.0±0.2 b	21.72
	2	1211.45		23.5±0.7 a		198.9±22.4 ab		738.2± 57.8 b		2889.6±116.5 a		64.7±0.1 b	
	4	1288.37		20.2±0.5 b		287.9±17.2 a		969.5± 22.9 a		2905.5±80.0 a		74.3±2.6 a	
	6	1463.75		16.4±0.1 c		153.8±9.7 c		526.3± 40.4 c		2690.4±95.3 a		59.2±3.0 b	
Overall Mean			37.39		26.76		31.74		28.70		27.62		15.20

Values within a column followed by different letters are significantly different at the 0.01 level for each cultivars

The highest value in MDA content in cv. '08-TR-003' was recorded as 25.2 µmol/g of fresh weight in 2-leaf-removal treatment. Increase percentage according to control was realized as 26.86%. In 'TR-3080' cultivar, the highest MDA content (32.1 µmol/g of fresh weight) was obtained from 4-leaf-removal treatment while it was 23.5 µmol/g of fresh weight in 2-leaf-removal treatment in cv. 'TARSAN-1018'. In all cultivars, MDA content decreased by increasing number of leaf removed. These results of our study were also corrected by Guo [22] who reported that MDA content decreased in defoliated plants (*Lycium chinense* Mill.) under salt stress conditions.

The activities of SOD were significantly ($P<0.01$) increased 35.21%, 24.74% and 35.28% in cvs. '08-TR-003', 'TR-3080' and 'TARSAN-1018' respectively, when leaf-removal treatments were performed. Patykowski and Kolodziejek [23] reported that the SOD activity was significantly ($P<0.05$) increased in *Viscum album* L. subsp. *album* plant leaves, when the host plants were defoliated depended on seasonal changing. The increase percentage according to control of CAT activity was observed as 21.94%, 5.19% and 28.98% which were statistically important at the level of 0.01, in cvs. '08-TR-003', 'TR-3080' and 'TARSAN-1018' respectively. Leaf-removal treatments gave rise to higher APX activities in all cultivars compared to control in which no leaf removed. Increase percentage in APX activities in cvs. '08-TR-003', 'TR-3080' and 'TARSAN-1018' were recorded as 17.93%, 27.11% and 37.83%, respectively. Results showed that GR activity increased as 18.82% in leaf-removal treatment compared to no-leaf-removal in '08-TR-003' cultivar. The highest GR activity was recorded in 6-leaf-removal treatment as 62.0 unit/min./mg fresh weight while the lowest was 49.5 in 2-leaf-removal treatment. The highest GR activity in 'TR-3080' cultivar was noted as 65.4 unit/min./mg fresh weight in 2-leaf-removal treatment while the lowest was recorded as 46.9 unit/min./mg fresh weight in 'TR-3080' cultivar. In cv. 'TARSAN-1018', the highest GR activity was observed in 4-leaf-removal treatment as 74.3 unit/min./mg fresh weight. The lowest value in GR activity was realized as 59.2 unit/min./mg fresh weight in 6-leaf-removal treatment.

Plantal crude oil need of Turkey was realized as 1 630 000 ton in 2014 [24]. Turkey's crude oil deficit was 835 000 ton in the same year [25]. 795 000 ton crude oil was obtained from sunflower in 2014. Turkey paid 1194 dollars for the importation of 1 ton plantal crude oil in 2014. That means that Turkey paid 996 990 000 dollars for importation of 835 000 ton crude oil deficit [25]. In Turkey, sunflower was planted in 5 524 651 decare, obtained 1 480 000 ton oil seed in 2014. And seed yield was 269.0 kg da^{-1}, crude oil yield was realized as 143.9 kg da^{-1} in the same year [26].

According to the results, when the production method described in our study based on leaf-removal from the plant in "Star-Shaped Head Stage" is used, seed yield per decare will rise to 297.1 kg from 269.0 kg with the increase of 10.46%. And crude oil yield per decare will also rise to 165.5 kg from 143.9 kg with the increase of 14.98%. This shows that our crude oil production from sunflower will be 914 329 ton (165.5 kg x 5 524 651 decare). In other words, crude oil production of Turkey will increase 119 329

ton (914 329 ton – 795 000 ton) when the method in the current study is performed. When it is thought that 1194 dollars was paid for the importation of 1 ton crude oil in 2014, 142 478 826 (119 329 ton x 1194 dollars) dollars income will be provided in Turkey.

CONCLUSION

By the current study, it was shown that crop production could be increased by stimulating plants physiologically. In our preliminary experiments, it was determined that photosynthetic activity could be increased by leaf-removal at a certain number. It was revealed that seed and crude oil yields in sunflower could be increased significantly by the method described in the current study. And the method described in the current study could easily be used in other crops in order to increase yield.

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