



The Effect of Inorganic Fertilizer and Biofertilizer Applications on Some Quality and Biochemical Properties of Safflower (*Carthamus tinctorius* L.)

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Abstract: This study was carried out in irrigated conditions during the summer growing season of 2020 and 2021 to determine the effects of biofertilizer applications and inorganic fertilization on some quality and biochemical properties of safflower (*Carthamus tinctorius* L.) in Van ecological conditions. The experiment was set up as a randomized block design in 3 replicates at the Faculty of Agriculture, Van Yüzüncü Yıl University. The mixture of five different biofertilizers (*Frateuria aurantia* (B₁), *Bacillus megaterium* (B₂), *Azospirillum lipoferum* (B₃), *Chlorella saccharophila* (B₄), and a mixture of *Lactobacillus casei* + *Rhodopseudomonas palustris* + *Saccharomyces cerevisiae* + *Lactococcus lactis microorganisms* (B₅)) different NP (nitrogen+phosphorus) fertilizer doses (control, 100% NP (NP₁₀₀) as full dose (optimum) 15 kg of pure nitrogen (Ammonium sulfate (21%) and 8 kg of pure phosphorus (TSP (42%)) per decare); % 7.5 kg of pure nitrogen (Ammonium sulfate (21%) and 4 kg of pure phosphorus (TSP (42%)) were applied as 50 NP (NP₅₀) reduced dose per decare. Some quality and biochemical Parameters including petal yield, crude oil rate, crude oil yield, total dyestuff ratio, total phenolic substance content, total flavonoid substance content and total antioxidant activity were measured. According to the results of the research; In both experimental years, the best results for crude oil yield and petal yield were obtained from NP₁₀₀ applications, while the best results for total flavonoid substance content and total antioxidant activity were obtained from NP₀ applications. B₄ biofertilizer applications for crude oil ratio, B₁ biofertilizer applications for petal yield, and B₅ biofertilizer applications for total phenolic content were the biofertilizer applications with the best results in both years.

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1. Introduction

Due to the insufficient production and high cost of animal fats, there has been a tendency for vegetable oils, and vegetable oils have had a larger share in human nutrition than animal fats. Oil plants have great importance in both human and animal nutrition due to the protein, mineral substance, fat, carbohydrate, and vitamins they contain (Kolsarıcı et al., 2015). The amount of energy that the consumed foods provide to the body varies. It is known that the energy provided by the same amount of

fat is more than twice as much as protein and carbohydrate (Bahar, 1999). Oils; It is used as a raw material in many industrial establishments such as cleaning, construction, medicine, cosmetics, plastic, and biodiesel production (Arioğlu, 2016). It was reported that the total production amount of oil crops worldwide in 2020 was 610.1 million tons and increased by 3.8% compared to 2019 (FAO, 2021). It is reported that the 2020 oil crops production area in our country is around 8.9 million decares, and the production amount is around 3.5 million tons (TUIK, 2021). The production area and quantities of the first five provinces, respectively, where safflower cultivation is carried out in our country, are given in Table 1.

Safflower, which is known to have originated in South Asia, spread to the Middle East and Mediterranean coasts, and has been cultivated in India, Egypt, Japan, Iran, and China and it is a species with a history of approximately 3500 years (Johnson et al., 2001). Safflower was first brought to our country by the Turks who immigrated from Central Asia, and its cultivation started in the 1940s. Safflower breeding was first started in the 1930s (Berber, 2007). Safflower, known as false saffron, zaferan, or painter's saffron, is an annual plant with wide leaves and flowers in colors such as yellow, orange, red, white, and cream, with thorny or thornless forms and with a height of up to 100 cm. It is known that the thorny forms have a higher oil rate and lower flower amount than the thornless forms. Safflower is a plant suitable for growing in arid regions, containing 30-50% oleic and linoleic acids in its seeds (Babaoğlu, 2005).

Table 1. According to TUIK 2020 data, the production area and quantities of the first five provinces where safflower cultivation is carried out, respectively, in our country

Provinces	Production area (decare)	Amount of production (kg decare ⁻¹)
Ankara	40 479	5 675
Muş	18 200	3 270
Aksaray	17 957	2 483
Konya	13 648	1 812
Gümüşhane	7 000	921

HSYA (Hydroxy Safflor Yellow A) constitutes most of the color pigments of the safflower flower. Since HSYA determines the majority of the therapeutic effects of safflower flowers, it is especially included in the Chinese pharmacopoeia (Ao et al., 2018).

It is reported that HSYA amounts of flower samples of different safflower varieties obtained from many countries are 0.05 mg-14.99 mg g⁻¹. It is reported that the environment in which the safflower plant grows, the color scale of the flower, and the harvest time are among the most important environmental factors affecting the HSYA rate (Zhao et al., 2020). It is reported that HSYA in safflower flowers decreases from dark colors to light colors (Xu et al., 2018), and the highest HSYA value is obtained in the morning hours, 4 or 5 days after the beginning of flowering (Tian et al., 2008).

Recently, it has been known that the colorants obtained from safflower flowers are used as natural colorants in the coloring of cosmetic products, face creams, shampoo, body lotion, and hair creams (Al-Snafi, 2015). Considering the frequently consumed beverages, foods such as candy, and pharmaceutical products such as syrup and tablets used in treatment, the importance of natural colorants is understood (Babaoğlu, 2005).

As a result of pharmacological studies, safflower flower extracts are used; In the protection of endothelial (inner surface cells of the vessels) cells, in the prevention of cerebral ischemia, dementia, parkinsonism, and traumatic brain damage from nervous system disorders, in acute lung inflammation, pneumonia and healing of wounds, which are among lung diseases, in coagulation, high blood pressure and heart enlargement from cardiovascular diseases. It is reported that it can be used in the prevention of circulatory failure, connective tissue growth, and tissue damage, which are liver diseases, and in the treatment of cancer disease and metabolic disorders (Ao et al., 2018).

Safflower seed contains less than 10% saturated fatty acids and more than 90% unsaturated fatty acids. It has been reported that palmitic and stearic fatty acids constitute the majority of saturated fatty acids in safflower seeds, while the majority of unsaturated fatty acids are composed of oleic (omega-9) from monounsaturated fatty acids and linoleic (omega-6) fatty acids from polyunsaturated fatty acids (Gürbüz, 1987). It is reported that safflower seeds contain 1.5-2.4% stearic fatty acid, 5.7-7.9% palmitic fatty acid, 13.5-17.6% oleic acid and 72.5-77.4% linoleic acid (Joshani et al., 2019). Safflower seeds

contain higher omega-6 fatty acids than olive, sunflower, rapeseed, corn, and hazelnut (Coşge et al., 2007).

Unsaturated fatty acids have important contributions to brain development, prevention of cardiovascular diseases, and strengthening of the body's defense system (Eseceli et al., 2006). In addition, unsaturated fatty acids are reported to be effective in increasing blood circulation and preventing congestion, reducing menstrual pains, lowering cholesterol levels, reducing insulin resistance and heart attack risk by causing an increase in the substance called adipopectin in the blood, and reducing obesity and hypertension (Nagao et al., 2003; Pischon et al., 2004; Guo, 2011).

Depending on the increase in the world population, the need for agricultural products and especially for plant production, is increasing day by day. Increasing crop production is mostly related to the use of synthetic fertilizers in sufficient quantities and at the right time. However, depending on the increase in the use of chemical fertilizers, the ecological balances deteriorated and the harmful aspects along with their benefits began to emerge, and the search for alternative fertilizers emerged. Biofertilizers, which are one of the leading alternative fertilizers, have recently started to be used in plant production due to their low cost, easy application, and being suitable for ecological agriculture. Microbial fertilizers (biofertilizers) are living organisms that contribute to the healthy growth and development of the plant in the root zone where it is applied, help reduce the damage caused by pathogens, facilitate the uptake of plant nutrients from the soil, and have a direct or indirect effect on reducing the damage caused to the plant by environmental stress conditions (Elnahal et al., 2022).

Determining the relationship of biofertilizers with the plant and determining their possible contributions to the plant is of great importance for the adoption and sustainability of organic agriculture. This study was carried out to determine the effects of different nitrogen and phosphorus combinations and environmentally friendly biofertilizer applications on some quality and biochemical parameters of the safflower plant, which has an important place in closing the oil deficit of our country in Van ecological conditions.

2. Material and Methods

The study was carried out in irrigated conditions in a summer cottage in the field crops trial area of Van Yüzüncü Yıl University Faculty of Agriculture in 2020 and 2021. In the study, as seed material, the registered oleic type safflower variety "Asol" in the spiny form obtained from Trakya Agricultural Research Institute was used.

According to Table 2, it is seen that the total precipitation in 2020 (336.8 mm) and the total precipitation in 2021 (206.2 mm) in the province of Van, where the research was conducted, are lower than the long term average (406.2 mm). It is seen that the average temperature of the 2020 trial year (10.76 °C) and the 2021 trial year's average temperature (11.73 °C) are higher than the long-term average (10.05 °C). It is seen that the average humidity rate in 2020 (59.16%), in which the experiment was conducted, was above LTA (Long-Term Average), while the average humidity rate in 2021 (53.29%) was below LTA.

According to the results of the analysis of the soil sample in which the experiment was conducted, it was determined that it was a calcareous, slightly alkaline reaction, unsalted, sufficient in potassium, low in phosphorus, and weak in terms of organic matter, according to both research years.

Biofertilizer promotes plant growth in Van ecological conditions (B₀: Control, B₁=*Frateruria aurentia*, B₂=*Bacillus megaterium*, B₃=*Azospirillum lipoferum*, B₄=*Chlorella saccharophilia* (microalgae), B₅=*Lactobacillus casei* + *Rhodospseudomonas palustris* + *Saccharomyces cereviscociae* + *Lactococcus lactis*) inorganic fertilization (control, 50% NP reduced dose =7.5 kg da⁻¹ pure (NH₄)₂SO₄; 4 kg da⁻¹ pure P₂O₅, 100% NP full dose=15 kg da⁻¹ pure (NH₄)₂SO₄; 8 kg da⁻¹ pure P₂O₅) and It was aimed to determine some quality and biochemical characteristics of Asol safflower variety by applying different combinations of these. This study was organized according to the "Divided Plots in Random Blocks Trial Design" with 3 replications. The experiment was planned with inorganic fertilizer (NP) doses to the main plots and biofertilizer to the sub-plots. The trial field was left for the winter after deep plowing was done in the autumn, and it was made ready for planting by making surface plowing just before planting in the spring.

Table 2. Average temperature (°C), total precipitation (mm) and average humidity (%) values for 2020, 2021, and long years of Van province where the experiment was conducted

Months	AT (°C)			TP (mm)			AH (%)		
	2020	2021	LTA	2020	2021	LTA	2020	2021	LTA
January	-2.5	-0.7	-2.5	43.8	13.0	33.2	74.5	67.1	66.7
February	-1.7	0.8	-1.5	79.9	12.9	31.5	77.1	73.3	67.2
March	4.9	3.7	2.8	44.3	39.9	47.7	72.5	67.0	65.4
April	8.6	11.7	8.4	51.8	5.0	57.4	65.4	48.8	59.3
May	14.5	16.7	13.4	27.8	20.2	45.3	54.0	46.4	55.1
June	19.3	21.6	18.8	13.7	0.2	16.4	44.4	32.0	47.1
July	23.0	24.2	22.7	17.6	4.6	6.9	46.4	38.4	42.3
August	21.6	23.5	22.9	10.0	1.4	5.3	44.5	38.0	40.5
September	20.1	18.8	18.3	5.6	6.3	20.4	41.3	40.6	43.9
October	13.3	12.3	12	1.8	50.2	48.2	53.0	51.0	57.3
November	6.7	7.0	5.1	12.8	23.1	48.8	65.4	69.6	64.2
December	1.4	1.2	0.2	27.7	29.4	45.1	71.5	67.3	67.5
Average	10.76	11.73	10.05				59.16	53.29	56.37
Total				336.8	206.2	406.2			

* Meteorological data of the experimental area were obtained from the Van Meteorology Regional Directorate.

LTA: Long-Term Average, TP: Total Precipitation, AT: Average Temperature, AH: Average Humidity.

In the experiment, a distance of 2m was left between the blocks and 1m between the plots. The research plots are 3m x 1.8m = 5.4m² in size, and each plot is arranged with 30 cm row spacing and 6 rows. The total area of the experiment was 13m x 49.4m = 642.2 m², and 54 parcels were included in the trial. The sowing process was carried out manually at a depth of 3 cm in the lines opened with the marker so that 3 kg of seeds per decare were used in both years. Biofertilizer applications, from biofertilizers per 1 kg of seeds (according to the manufacturer's instructions for use); Seeds in bacterial suspensions prepared as B₁ = 10 ml L⁻¹ distilled water, B₂ = 10 ml L⁻¹ distilled water, B₃ = 10 ml L⁻¹ distilled water, B₄ = 50 ml L⁻¹ distilled water and B₅ = 10 ml L⁻¹ distilled water, were added separately, and the coating process was carried out in this way.

Covered seeds were filtered and placed on blotting papers to dry in the shade for 30 minutes. Bacteria-covered seeds prepared for sowing were sown early in the morning to avoid the negative effects of sun rays on bacteria, and the seeds were immediately covered during sowing. In the rosette period, when the plants had 3-4 leaves (10-15 cm), thinning was done to 15 cm above the row. After thinning, biofertilizer solutions were prepared at the concentrations given above, and the prepared biofertilizer solutions were applied to the root zone of the plants, with an average of 125 ml per row in the plot. The application of Bio Fertilizer solutions to the plant root zone was made at sunset so that the biofertilizers would not be affected by light.

During the rosette period, when the plants had 3-4 leaves (10-15 cm), misfires were done on the row, with one plant in every 15 cm. In the experiment, 15 kg pure nitrogen (Ammonium sulfate (21%) and 8 kg pure phosphorus (TSP (42%)) as 100% NP full dose (optimum) per decare; 7.5 kg pure nitrogen per decare as 50% NP reduced dose (Ammonium sulfate (21%) and 4 kg of pure phosphorus (TSP (42%)) were applied (Tunçtürk, 2003). All of the phosphorus was given with the planting. Half of the nitrogen was given in the sowing and the other half in the plant elongation period. In both experimental years, the water needs of the plants were met with the sprinkler irrigation method due to insufficient rainfall after the plants emerged, during the plant elongation period, and in the pre-flowering period. After the plants germinated, weeds in the plots were removed by mechanical control, and hoeing was carried out before the plant elongation and flowering periods. Since disease or pest was not observed in the experiment, chemical control was not applied. Harvesting was done when the petals of the plants were completely dry, the grains turned white, and the leaves turned brown. All the measurements were

carried out on the remaining 2.4 m² (1.2m x 2m) area after the 6 rows forming the plot were excluded from the observation as a side effect of 50 cm from each side and the top of the row.

2.1. Petal yield (kg da⁻¹)

The total weight of the petals of the plants in one m² harvested from the plots was determined as kg da⁻¹ by weighing with an accuracy of 0.001 g (Kızıllı, 1997).

2.2. Crude oil ratio (%)

Crude oil was obtained by using hexane as a solvent in Soxhlet type extractors. The results were determined as % over dry matter (Tunçtürk, 2003).

2.3. Crude oil yield (kg da⁻¹)

Crude oil yield values were calculated as a result of simple mathematical multiplication of % crude oil ratio values with seed yield values obtained from the same plot (Tunçtürk, 2003).

2.4. Total dyestuff ratio (%)

Total dyestuff determination was determined by modifying the method developed by Harborne (1973) for polar flavonoids. For dyestuff extraction, 0.5 grams of flower samples were taken, 10 ml of 2M HCL (37%) was added and left in a heated shaker for 2 hours. 15 ml of ethyl acetate was added to each sample taken from the shaker, vortexed for 15-20 seconds, and then filtered with the help of a Büchner funnel. The obtained filtered samples were taken into tared petri dishes and kept at 40 °C for 2 days, the evaporation process was completed, the final weights of the petri dishes were determined, and the amount of dyestuff was calculated as %.

2.5. Total phenolic substance amount (mg GA 100⁻¹ g)

In the determination of the total amount of phenolic substance, the method developed by modifying the FolinCicaltea spectrophotometric method specified by (Obanda et al., 1997) was used. The Folin-Cicaltea solution was diluted at 1:3. Saturated sodium carbonate (35%) solution; 87.5 g of sodium carbonate was dissolved in distilled water, made up to 250 ml, and left overnight and then filtered. Gallic acid stock solution (500 µg ml⁻¹); was prepared by dissolving 50 mg of gallic acid in 100 ml of distilled water. Gallic acid working solution; Each 500 µg ml⁻¹ gallic stock solution was prepared in 5 ml measuring balloons as 9 separate solutions with concentrations ranging from 0-55 µg ml⁻¹. 1 ml of these solutions was taken and mixed with 1 ml of FolinCicaltea solution. After waiting for 5 minutes, 2 ml of sodium carbonate was added and shaken, and diluted with 2 ml of water. After this mixture was kept in the dark for 30 minutes, the absorbance value was read in the spectrometer at a wavelength of 700 nm. A calibration curve was obtained by graphing the absorbance values read against these different concentrations of gallic acid (r²= 97.47).

2.6. Total flavonoid substance amount (mg QE 100⁻¹ g)

Determination of the total amount of flavonoid substances; Total flavonoid substance determination was specified according to the method that (Quettier-deleu et al., 2000) developed. 2 ml of 2% AlCl₃ was added to 2 ml of extract and left in the dark for 1 hour at room temperature. The total flavonoid contents of the extracts were measured with a spectrophotometer at a wavelength of 415 nm by performing 2 parallel studies in each sample and calculated in mg QE 100⁻¹ g using the calibration curve prepared using standard quercetin.

2.7. Total antioxidant activity (mg TE g⁻¹)

Determination of total antioxidant activity; After weighing 2 g of safflower petal and adding 4 ml of methanol, the material passed through the homogenizer was centrifuged at 10000 rpm for 10 minutes, and the supernatant remaining on top was taken. Also, after preparing 300 mM acetate buffer (pH 3.6), 10 mmol L⁻¹ 2,4,6-tripyridyl-s-triazine (TPTZ) prepared by dissolving in 40 mM HCl and 20 mmol L⁻¹ FeCl₃.6H₂O solutions, FRAP reagent was prepared by mixing them at a ratio of 10:1:1,

respectively. The mixture prepared for the analysis of 2850 μL of FRAP reagent and ABTS (2,2-Azinobis 3-ethyl-benzothiazoline-6-sulfonic acid) on safflower petal was diluted 50 times with ethanol, then 150 μL of the sample was mixed and kept at room temperature for 30 minutes. The resulting ferrous tripyridyltriazine complex was measured at 593 nm in the spectrophotometer, and the results were reported as mg Trolox g^{-1} (Lutz et al., 2011). Trolox concentration range has been studied as 0-500 ppm.

2.8. Statistical analysis

The data obtained from the study were subjected to variance analysis with the Costat 6.303 package program according to the "Divided Plots in Random Blocks Experiment Pattern" in terms of separate years, and the averages obtained were grouped according to the LSD (0.05) multiple comparison test. However, in terms of united years, the data were subjected to variance analysis with the Costat 6.303 package program according to the "Divided Plots Trial Pattern in Random Blocks" and the averages obtained were grouped according to the LSD (0.05) multiple comparison test.

3. Results and Discussions

As a result of the study, it was determined that the effect of inorganic fertilizer \times biofertilizer interaction and inorganic fertilizer \times biofertilizer \times year interaction on all parameters examined was statistically significant according to the results of the analysis of variance performed in 2020, 2021, and the united years average.

3.1. Crude oil ratio

According to the results of the analysis of variance, the differences between years in terms of crude oil ratio were found to be statistically significant. The average crude oil rate was 31.09% in the 2020 research year and 25.24% in the second year (Table 3). It is thought that the precipitation falling in the vegetation period of 2020 is higher than the precipitation in the vegetation period of 2021, causing the average oil rate to increase significantly. Öztürk et al. (2008) reported that crude oil ratios of safflower cultivars were higher in a year with high annual precipitation. The effect of inorganic fertilizer applications on the crude oil ratio was not statistically significant in 2020, 2021, and the united years. The crude oil rate was found within the range of 30.19-31.55% in 2020, 24.71-25.86% in 2021, and 27.45-28.70% in united years averages (Table 3). In previous studies. It has been reported that the effect of different nitrogen doses on the crude oil ratio in safflower is statistically insignificant (Akış, 2013; Buçak, 2019; Ünsal, 2020), and NP applications do not cause a significant effect (Çelik, 2017; Demir and Karaca, 2018).

The effect of biofertilizer applications on the crude oil ratio was not statistically significant according to the united averages of the two years with the 2020 and 2021 trial years. Crude oil ratio average values were determined as 30.27-31.82% in 2020, 24.20-26.25% in 2021, and 27.29-29.04% according to the united averages of the two years (Table 3).

3.2. Crude oil yield

According to the results of the analysis of variance, the differences between years in terms of crude oil yield were found to be statistically significant. The average crude oil yield was 56.39 kg da^{-1} in the first year of the study and 41.01 kg da^{-1} in the second year (Table 3). The effect of inorganic fertilizer applications on crude oil yield was found to be statistically significant according to 2020, 2021, and united averages of two years. According to the average of 2020 and 2021 trial years and united years, the highest crude oil yield values were obtained from NP₁₀₀ applications with 74.45-49.66-62.06 kg da^{-1} , respectively. According to the average of 2020 and united years, the lowest values were determined from the control applications with 41.09-38.56 kg da^{-1} respectively. Compared to the 2021 average, the lowest crude oil yield of 36.02 kg da^{-1} was determined from the control applications and was statistically in the same group with the NP₅₀ applications (Table 3). It has been reported that crude oil yield in safflower increases in direct proportion to nitrogen doses but is not affected by phosphorus doses (Sezer, 2010; Karaca, 2017).

The effect of biofertilizer applications on crude oil yield was found to be statistically significant compared to the averages of 2020, 2021, and the united years. Compared to 2020, the highest crude oil

yield was achieved in B₅ applications with 65.05 kg da⁻¹, and the lowest crude oil yield was achieved in control applications with 44.12 kg da⁻¹. The highest crude oil yield in 2021 was obtained from B₁ applications with 46.98 kg da⁻¹, and the lowest value was obtained from B₃ applications with 24.29 kg da⁻¹. According to the united years' average, the highest crude oil yield of 53.28 kg da⁻¹ was determined from B₅ applications and was in the same statistical group with B₁, B₂, and B₄ applications. The lowest crude oil yield was determined from the control applications with 40.86 kg da⁻¹ and it was in the same statistical group with the B₃ applications. (Table 3). It is known that potassium accumulation occurs in the soil as a result of the activity of the bacterium *Frateuria aurantia* (B₁), which is effective in dissolving potassium in the soil and making it useful (Salem, 2020; Şelem et al. 2021). The increase in potassium in the soil causes an increase in the seed fullness and seed yield in the generative phase of the plant. It has been reported that crude oil yield increases in sunflowers due to increased potassium doses (Altınparmak, 2016; Yağmur and Okur, 2017). It is reported that the *Rhodopsudomonas palustris* bacterium (bacteria found in B₅ biofertilizer) fixes nitrogen to the soil, and accordingly, the soil is enriched with nitrogen (Sakpirom et al., 2019). It has been reported that the fixed oil yield of black cumin increases due to increasing nitrogen and potassium doses (Kızılyıldırım and Gedik, 2021), and the crude oil yield increases due to the increased nitrogen dose in sunflower (Yıldız, 2014). As a result of our study, it is thought that the increase of nitrogen and potassium minerals in the soil due to B₁ and B₅ applications and, consequently, the crude oil yield may have increased, as supported by the literature.

Table 3. Crude oil ratio (%), crude oil yield (kg da⁻¹), petal yield (kg da⁻¹), and total dyestuff ratio (%) values of different inorganic fertilizer doses and biofertilizer applications in safflower in 2020, 2021, and comined years

İF	COR (%)			COY (kg da ⁻¹)			PY (kg da ⁻¹)			TDR (%)		
	2020	2021	UY	2020	2021	UY	2020	2021	UY	2020	2021	UY
NP ₀	31.53	25.86	28.70	41.09 ^c	36.02 ^b	38.56 ^c	14.94 ^b	10.08 ^c	12.51 ^b	2.83 ^a	2.08	2.46 ^a
NP ₅₀	31.55	25.14	28.35	53.64 ^b	37.35 ^b	45.49 ^b	17.61 ^a	11.89 ^b	14.75 ^a	2.67 ^c	2.11	2.39 ^b
NP ₁₀₀	30.19	24.71	27.45	74.45 ^a	49.66 ^a	62.06 ^a	18.01 ^a	13.02 ^a	15.52 ^a	2.78 ^b	2.05	2.41 ^b
LSD (0.05)	ns	ns	ns	7.28	7.04	4.20	1.64	1.05	0.81	0.02	ns	0.02
B	2020	2021	UY	2020	2021	UY	2020	2021	UY	2020	2021	UY
B ₀	30.89	25.35	28.12	44.12 ^c	37.60 ^{bc}	40.86 ^b	15.74 ^c	9.76 ^c	12.75 ^d	2.68 ^{cd}	1.95 ^c	2.32 ^b
B ₁	30.27	24.31	27.29	57.57 ^{ab}	46.98 ^a	52.28 ^a	20.38 ^a	13.38 ^a	16.88 ^a	2.78 ^{a-c}	2.09 ^b	2.44 ^a
B ₂	31.81	26.22	29.01	57.52 ^{ab}	43.18 ^{ab}	50.35 ^a	15.99 ^{bc}	10.16 ^{bc}	13.07 ^d	2.75 ^{b-d}	2.21 ^a	2.47 ^a
B ₃	30.77	24.20	27.48	54.61 ^b	24.29 ^c	44.45 ^b	15.45 ^c	11.87 ^{ab}	13.66 ^{cd}	2.82 ^{ab}	2.10 ^b	2.46 ^a
B ₄	31.82	26.25	29.04	59.49 ^{ab}	42.49 ^{ab}	50.99 ^a	17.10 ^b	11.62 ^{ab}	14.36 ^{bc}	2.89 ^a	2.07 ^b	2.48 ^a
B ₅	30.99	25.09	28.04	65.05 ^a	41.50 ^{ab}	53.28 ^a	16.46 ^{bc}	13.20 ^a	14.83 ^b	2.65 ^d	2.08 ^b	2.36 ^b
LSD (0.05)	ns	ns	ns	7.09	5.70	4.45	0.99	1.76	0.99	0.11	0.08	0.06
CV (%)	3.09	9.31	6.52	11.54	14.44	13.73	5.45	15.68	10.42	4.11	4.34	4.27
YA	31.09	25.24	28.16	56.39	41.01	48.70	16.85	11.66	14.25	2.76	2.08	2.42

*There is no statistical difference between the values in the same column and written with the same lowercase letters.

LSD (P<0.05), CV: Coefficient Value, YA: Year Average, İF: İnorganic Fertilizer, NP: Nitrogen + Phosphorus, NP₀: Control, NP₅₀: Half dose, NP₁₀₀: Full dose, B: Biofertilizer, B₀: Control, B₁: *Frateuria aurantia*, B₂: *Bacillus megaterium*, B₃: *Azospirillum lipoferum*, B₄: *Chlorella saccharophila*, B₅: *Lactobacillus casei* + *Rhodopsudomonas palustris* + *Saccharomyces cerevisiae* + *Lactococcus lactis*. ns: non significant

COR: Crude Oil Ratio, COY: Crude Oil Yield, PY: Petal Yield, TDR: Total Dyestuff Ratio, UY: United Years.

3.3. Petal yield

According to the results of the analysis of variance, the differences between years in terms of petal yield were found to be statistically significant. The average petal yield was 16.85 kg da⁻¹ in the 2020 year and 11.66 kg da⁻¹ in the second year (Table 3). The effect of inorganic fertilizer applications on petal yield was found to be statistically significant in 2020, 2021, and united years. According to the average of 2020, 2021, and the united years, the petal yield values obtained from the NP₁₀₀ applications were better than the other applications with 18.01-13.02-15.52 kg da⁻¹ respectively. The lowest petal values were determined from NP₀ applications with 14.94-10.08-12.51 kg da⁻¹ according to 2020, 2021, and united years' average ranking (Table 3). It has been reported by various researchers that there is an increase in flower yield due to increased nitrogen applications in safflower (Amırgai and Koç, 2016;

Andırman and Karaaslan, 2021), while phosphorus applications increase flower yields in studies conducted on different plants (Çavuşoğlu, 2015; Muktar, 2021).

The effect of biofertilizer applications on petal yield was found to be statistically significant compared to the experimental years of 2020, 2021, and united years. According to the average of 2020 and united years, the highest petal yield was determined from B₁ applications with 20.38-16.88 kg da⁻¹ respectively. In 2021, the highest petal yield with 13.38 kg da⁻¹ was obtained from B₁ applications and was in the same statistical group with B₅ applications. According to the data of 2020, the lowest petal yield was determined at 15.45 kg da⁻¹ from B₃ applications, and no statistical difference was observed between with control applications. In 2021, the lowest petal yield was obtained from B₀ applications with 9.76 kg da⁻¹. According to the united years' average, the lowest petal yield was obtained from B₀ applications with 12.75 kg da⁻¹, and there was no statistical difference between B₂ applications (Table 3).

3.4. Total dyestuff ratio

According to the results of the analysis of variance, the differences between years in terms of total dyestuff ratio were found to be statistically significant. The average total dyestuff rate was 2.76% in the 2020 year and 2.08% in the second year (Table 3). While the effect of inorganic fertilizer applications on the total dyestuff ratio was statistically significant compared to the averages of 2020 and the united years, it was found to be insignificant in 2021. According to the average of 2020 and united years, the highest total dyestuff ratio was obtained from NP₀ applications with 2.83-2.46%, respectively. The lowest total dyestuff ratio in 2020 was obtained from NP₅₀ applications with 2.67%. According to the united years average, the lowest total dyestuff ratio was determined as 2.39% in NP₅₀ applications, and it was in the same statistical group with NP₁₀₀ applications. The total dyestuff ratio in 2021 was in the range of 2.05-2.11% (Table 3). The reason why the highest dyestuff ratio is obtained from the control applications, according to the average of 2020 and the united years, is that the plant is stressed by showing a mineral substance deficiency due to the lack of inorganic fertilizer. As a result, the amount of flavonoid substances, which increased in many plants under stress, caused an increase in the amount of dyestuff in the flavonoid structure in our study and the corresponding increase in the total dyestuff ratio. Studies have been conducted on the increase of various substances in the flavonoid structure due to mineral substance deficiency in various plants (Heimler et al., 2017; Mokgehle et al., 2017; Şahin, 2018).

The effect of biofertilizer applications on the total dyestuff ratio was found to be statistically significant in the averages of 2020, 2021, and two years. In 2020, the highest total dyestuff ratio was obtained from B₄ applications with 2.89%, and the lowest value was obtained from B₅ applications with 2.65%. In 2021, the highest total dyestuff ratio was obtained from B₂ applications with 2.21%, and the lowest value was obtained from B₀ applications with 1.95%. According to the united years average, the highest total dyestuff ratio with 2.48% was determined from B₄ applications, but it was statistically in the same group with B₁, B₂, and B₃ applications. The lowest value was determined from B₀ applications with 2.32%, and there was no statistical difference between with B₅ applications (Table 3). It is estimated that microalgae contain minerals, hormones, and vitamins necessary for plants (Piwowar and Harasym, 2020) and can activate many metabolic activities, such as pigment production. Accordingly, we think that the rate of dyestuff may have increased with the increase in color pigments.

3.5. Total phenolic substance amount

According to the results of the analysis of variance, the differences between years in terms of the total amount of phenolic substances were found to be statistically significant. In the first research year, the average total amount of phenolic substances was found 122.76 mg GAE 100⁻¹ g, and in the second year, it was 79.48 mg GAE 100⁻¹ g (Table 4). The effect of inorganic fertilizer applications on the total amount of phenolic substances was not found to be statistically significant averages of 2020, 2021, and two years. The total amount of phenolic substances range in 2020 was determined as 121.13-124.52 mg GAE 100⁻¹ g, in 2021, it was 75.33-86.67 mg GAE 100⁻¹ g, and according to the averages of two years, it was determined between 98.78-104.66 mg GAE 100⁻¹ g (Table 4).

The effect of biofertilizer applications on the total amount of phenolic substances was found to be statistically significant in 2020, 2021, and united years. In 2020, the highest total phenolic substance content was determined with 128.21 mg GAE 100⁻¹ g from B₅ applications and was included in the same

statistical group with B₃ applications. The lowest value was obtained from B₂ applications with 112.33 mg GAE 100⁻¹ g. In 2021, the highest total phenolic substance amount was determined from B₅ applications with 86.38 mg GAE 100⁻¹ g, and the lowest value was determined from B₀ applications with 72.86 mg GAE 100⁻¹ g. According to the averages of two years, the highest total phenolic substance content was determined from B₅ applications with 107.29 mg GAE 100⁻¹ g. the lowest total amount of phenolic substance was determined in united years from B₀ applications with 97.25 mg GAE 100⁻¹ g, and it was in the same statistical group with B₂ applications (Table 4). According to the trial years and united years, it is seen that B₅ applications are more effective than other biofertilizers in increasing the total phenolic content values (Table 5). It has been reported that *Rhodopsudomonas sp.* increases secondary metabolites in phenolic and flavonoid structures in different plants (Lee et al., 2009), and *Rhodopseudomonas palustris* bacteria application in cucumber grown under salt stress is effective in reducing salt stress by increasing phenolic compounds (Ge and Zhang, 2019).

Table 4. Average total phenolic substance amount (mg GAE 100⁻¹ g), total flavonoid substance amount (mg QE 100⁻¹ g), and total antioxidant activity (mg TE 100⁻¹ g) values of biofertilizer applications with different inorganic fertilizer doses in safflower between 2020-2021 and two years

İF	TPSA (mg GAE 100 ⁻¹ g)			TFSA (mg QE 100 ⁻¹ g)			TAA (mg TE g ⁻¹)		
	2020	2021	UY	2020	2021	UY	2020	2021	UY
NP ₀	122.65	86.67	104.66	11.87 ^a	9.96 ^a	10.78 ^a	11.95	9.05	10.50 ^a
NP ₅₀	124.52	75.33	99.92	7.81 ^b	8.84 ^b	8.33 ^c	10.82	8.17	9.50 ^b
NP ₁₀₀	121.13	76.44	98.78	10.89 ^a	8.71 ^b	9.80 ^b	10.21	8.20	9.20 ^b
LSD (0.05)	ns	ns	ns	1.29	0.55	0.58	ns	ns	0.84
B	2020	2021	UY	2020	2021	UY	2020	2021	UY
B ₀	121.63 ^b	72.86 ^c	97.25 ^c	8.36 ^b	8.32 ^c	8.34 ^c	10.65 ^{bc}	7.96 ^b	9.30 ^{cd}
B ₁	122.39 ^{ab}	77.03 ^{bc}	99.71 ^{bc}	9.08 ^b	8.16 ^c	8.62 ^{bc}	9.77 ^c	8.31 ^{ab}	9.04 ^d
B ₂	112.33 ^c	79.35 ^{a-c}	95.84 ^c	8.83 ^b	8.64 ^{bc}	8.73 ^{bc}	10.71 ^{bc}	8.64 ^a	9.67 ^{bc}
B ₃	125.58 ^a	80.07 ^{a-c}	102.82 ^{ab}	12.44 ^a	10.94 ^a	11.69 ^a	11.75 ^{ab}	8.64 ^a	10.19 ^{ab}
B ₄	124.45 ^{ab}	81.20 ^{ab}	103.82 ^{ab}	9.19 ^b	9.16 ^b	9.18 ^b	12.30 ^a	8.56 ^a	10.43 ^a
B ₅	128.21 ^a	86.38 ^a	107.29 ^a	13.25 ^a	9.27 ^b	11.26 ^a	10.78 ^{bc}	8.74 ^a	9.76 ^{bc}
LSD (0.05)	5.73	7.87	4.76	1.20	0.73	0.69	1.11	0.56	0.61
CV (%)	4.72	10.28	7.07	8.56	8.42	10.76	10.77	6.92	9.45
YA	122.76	79.48	101.12	10.19	9.08	9.63	10.99	8.47	9.73

*There is no statistical difference between the values in the same column and written with the same lowercase letters.

LSD (P<0.05), CV: Coefficient Value, YA: Year Average, İF: İnorganic Fertilizer, NP: Nitrogen + Phosphorus, NP₀: Control, NP₅₀: Half dose,

NP₁₀₀: Full dose, B: Biofertilizer, B₀: Control, B₁: *Frateruria aurantia*, B₂: *Bacillus megaterium*, B₃: *Azospirillum lipoferum*, B₄: *Chlorella saccharophila*, B₅: *Lactobacillus casei* + *Rhodopseudomonas palustris* + *Saccharomyces cerevisiae* + *Lactococcus lactis*, ns: non significant

TPSA: Total Phenolic Substance Amount, TFSA: Total Flavonoid Substance Amount, TAA: Total Antioxidant Activity, UY: United Years.

3.6. Total flavonoid substance amount

According to the results of the analysis of variance, the differences between years in terms of the total amount of flavonoid substances were found to be statistically significant. In 2020, the average total amount of flavonoid substance was found to be 10.19 mg QE 100⁻¹ g, and in the second year 9.08 mg QE 100⁻¹ g (Table 4). The effect of inorganic fertilizer applications on the total amount of flavonoid substances was determined to be statistically significant compared to the averages of 2020, 2021 and united years. In 2020, the highest total amount of flavonoid substance was observed in NP₀ applications with 11.87 mg QE 100⁻¹ g, and it was in the same statistical group with NP₁₀₀ applications. The lowest value was obtained in NP₅₀ applications with 7.81 mg QE 100⁻¹ g. According to the 2021 and united years average, the highest total flavonoid substance amounts were determined in NP₀ applications with 9.96-10.78 mg QE 100⁻¹ g, respectively. In 2021, the lowest total flavonoid substance amount was determined with 8.71 mg QE 100⁻¹ g from NP₁₀₀ applications and was statistically in the same group with NP₅₀. According to the united years' average, the lowest total flavonoid substance content was determined with 8.33 mg QE 100⁻¹ g from NP₅₀ applications (Table 4). It has been reported that flavonoids increase under stress situations such as limited water, high amounts of salt, and insufficient nutrients (AlKahtani et al., 2021). When our study results are examined, it is seen that the total flavonoid amounts are higher in applications without nitrogen and phosphorus fertilization than in other applications due to insufficient nutrients in the soil.

The effect of biofertilizer applications on the total amount of flavonoid substances was found to be statistically significant according to 2020, 2021, and united years. In the 2020 year, the highest total flavonoid substance content was determined with 13.25 mg QE 100⁻¹ g from B₅ applications and was in the same statistical group as B₃ applications. The lowest total flavonoid substance amount in 2020 was obtained from B₀ applications with 8.36 mg QE 100⁻¹ g, and there was no statistical difference between B₁, B₂, and B₄ applications. In the 2021 year, the highest total flavonoid substance amount was determined from B₃ applications with 10.94 mg QE 100⁻¹ g, the lowest total flavonoid substance amount was determined from B₁ applications with 8.16 mg QE 100⁻¹ g, and it was in the same statistical group with B₀ applications. According to the united years' average, the highest total amount of flavonoid substance was obtained from B₃ applications with 11.69 mg QE 100⁻¹ g, and it was in the same statistical group as B₅. The lowest total flavonoid substance amount in united years average was obtained from B₀ applications with 8.34 mg QE 100⁻¹ g (Table 4). *Azospirillum* sp. It is reported that flavonoid application causes an increase in flavonoid compounds in rice (Chamam et al., 2013). It is reported that the application of *Azospirillum lipoferum* in chickpea increased phenolic and flavonoid compounds (El-Esawi et al., 2019). The literature results are in agreement with our findings.

3.7. Total antioxidant activity

According to the results of the analysis of variance, the differences between years in terms of total antioxidant activity were found to be statistically significant. The average total antioxidant activity was found to be 10.99 mg TE g⁻¹ in 2020 and 8.47 mg TE g⁻¹ in the second year of the study (Table 4). While the effect of inorganic fertilizer applications on total antioxidant activity was not statistically significant in 2020 and 2021, it was found to be significant compared to the averages of the united years. The total antioxidant activity range for 2020 and 2021 was determined as 10.21-10.95 and 8.17-9.05 mg TE g⁻¹, respectively. In the united years, the highest total antioxidant activity value was determined in NP₀ applications with 10.50 mg TE g⁻¹. The lowest total antioxidant activity value in united years was observed in NP₁₀₀ applications with 9.20 mg TE g⁻¹, and it was in the same statistical group with NP₅₀ applications (Table 4).

The effect of biofertilizer applications on total antioxidant activity was found to be statistically significant compared to the experimental years of 2020 and 2021 and the averages of two years. According to the 2020 and united years average, the highest total antioxidant activity was obtained from B₄ applications with 12.30-10.43 mg TE g⁻¹, respectively, and the lowest total antioxidant activity was obtained from B₁ applications with 9.77-9.04 mg TE g⁻¹, respectively. The total antioxidant activity value of 8.74 mg TE g⁻¹ obtained from B₅ applications in 2021 gave better results than other applications. The lowest value was determined in B₀ applications with 7.96 mg TE g⁻¹ in 2021 (Table 4). Secondary metabolite production in plants is directly related to the macronutrients in the soil (Zhang et al., 2017), and it is reported that microalgae applications increase the uptake of micro and macro nutrients in the soil (Gatamaneni et al., 2020). It has been reported that microalgae application in basil increases antioxidant activity (Hristozkova et al., 2017), while growth and secondary metabolites increase due to microalgae applications in different studies (Garcia-Gonzalez and Sommerfeld, 2016; Wuang et al., 2016).

Conclusion

This research, which was carried out in Van ecological conditions in the summer growing season of 2020 and 2021, was carried out to determine the effects of biofertilizer applications and inorganic fertilization on some quality and biochemical properties of safflower (*Carthamus tinctorius* L.). According to the average of both trial years and united years, it was determined that the petal yield and crude oil yield increased depending on the increase in NP doses, and the crude oil ratio and the total phenolic substance content were not affected. Depending on the increase in NP doses, it was determined that there were decreases in parameters such as total dyestuff content, total flavonoid substance content, and total antioxidant activity in 2020, 2021, and united years.

According to the average of 2020, 2021, and united years, it was determined that the biofertilizer applications had a statistically significant effect on all parameters except the crude oil ratio. Considering the average values of the trial years and united years, more positive results were obtained in NP fertilizer doses and biofertilizer applications compared to the control plots. It has been observed that NP

fertilization gives better results than biofertilizer applications for many parameters. It is estimated that the fact that the highest values in the investigated parameters were obtained from different biofertilizer agents according to the years, different soil temperatures, climatic conditions, and partially small changes in soil pH may be due to the inability to reach the optimum level in bacterial colonization. Likewise, it is thought that the values that vary according to the years in the examined parameters may be caused by climatic conditions such as precipitation, temperature, and humidity.

As a result, It has been concluded that biofertilizer applications cannot replace NP applications and will be insufficient when applied alone, but the best results can be obtained when applied together with NP. It was determined that the most suitable nitrogen-phosphorus (NP) dose to be recommended for safflower cultivation in Van conditions was NP₁₀₀. B₁ (*Frateruria aurantia*) for petal yield, B₅ (*Lactobacillus casei* + *Rhodopseudomonas palustris* + *Saccharomyces cerevisiae* + *Lactococcus lactis*) for crude oil yield and total phenolic content, and B₄ (*Chlorella saccharophila*) for other parameters are the best biofertilizer agents to be recommended.

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