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BENEFITS OF MICROBIAL FERTILIZERS APPLIED AT DIFFERENT GROWTH STAGES ON OIL CONTENT AND QUALITY PROPERTIES OF PEANUT

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ABSTRACT

Microbial fertilizer helps the plants grow healthy and stay strong due to including fertilizer and beneficial microorganisms. The purpose of the study was to determine the effects of microbial fertilizers applied in different growth stages on oil content, fatty acid composition and oil quality of peanut (*Arachis hypogaea* L.). The study was conducted in Osmaniye/Turkey in 2019 and 2020 according to RCBD arranged in split-plots design with 3 replications. The highest oil contents were observed in 150 cc da^{-1} (53.07%) and beginning bloom + full bloom (53.95%) treatments in studied years. For total unsaturated fatty acid compositions, the highest values were obtained in full bloom (79.49%) and 100 cc da^{-1} (79.99%) treatments. The highest O/L ratio were observed in full bloom and 100 cc da^{-1} treatments as 1.72 and 1.68, respectively according to mean of 2-year values. Similar results were seen in IV, and these treatments came forefront with the values 93.83 and 94.52, respectively. As a results of the study, it was concluded that (i) microbial fertilizers had the positive effects on oil content and quality parameters of peanut, (ii) the treatment 100 cc da^{-1} applied in full bloom may be the optimum selection.

Key words: Iodine value, oleic/linoleic acid ratio, peanut, quality

INTRODUCTION

Groundnut or peanut (*Arachis hypogea* L.) is a member of the *Fabaceae* family, and a valuable product due to high quality oil and protein content. Its seed contains about 55% oil and 25% protein depending on market types and growing conditions. Oleic (C18:1) and linoleic (C18:2) acids are the predominant fatty acids with about 80% of the total fatty acid composition. The other important acids are stearic (C18:0), palmitic (C16:0), arachidic (C20:0), lignoceric (C24:0) and behenic (C22:0) acids which are all saturated fatty acids unlike oleic and linoleic acids (Yol and Uzun, 2018; Sahin et al., 2022).

High oleic acid content reduces cardiovascular disease risk, supplies an extended shelf life for peanut-derived products, and decreases low-density lipoprotein cholesterol levels (Mora-Escobedo et al., 2015; Yol and Uzun, 2018; Yilmaz, 2022). Increasing the oleic to linoleic acid (O/L) ratio and decreasing the iodine value (IV) provide a stability for oil and long shelf life for products made with groundnut (Yol et al., 2017).

Microbial fertilizers (MF), also known as organic fertilizers, are environment friendly because they mainly consisting of food and agricultural waste (animal manure, straw etc.) and beneficial microorganisms. They improve the soil aggregate structure, increase the soil fertility hence promote crop growth and resistance, and provide more nutrition to soil (Ozturk and Yildirim, 2013; Yan et al., 2021; Zhou et al., 2021a). MF, the eco-friendly fertilizer, are rich source of different types of living microbial cells, and slowly release nutrients continuously, thus increasing fertility and soil productivity (Dayan et al., 2018; Chen et al., 2022).

In a field experiment conducted by Dayan et al. (2018), MF and plant activators increased the yield and quality of pumpkin. Dizajeyekan et al. (2021) also reported that biofertilizers increased the seed oil percentage and fatty acid contents of rapeseed. Similar results were also seen in some research made by Oliviera et al. (2021) and Han et al. (2022). Oliviera et al. (2021) conducted an experiment with lettuce and observed that MF improved the plant productivity and soil properties. Han et al. (2022) reported that the crude fat content was increased by 22.97% in Elymus nutans plant compared to control. It was reported that in China, MF have been widely used in the cultivation of some medicinal materials, which not only improve the structure of soil, but also promote the quality and yield of medicinal materials (Zhou et al., 2021b).

As mentioned above, the MF could play a significant role in sustainable agriculture. The aim of the study was to determine the effects of MF doses and growth stage of peanut (*Arachis hypogea* L.) grown under Mediterranean climate region on oil content and quality properties.

MATERIALS AND METHODS

Materials

The present study was conducted out in the experimental fields of Osmaniye Oil Seed Research Institute (37°07'39.7"N; 36°12'03.8"E; 70 m) in Eastern Mediterranean of Turkey during the main growing seasons of 2019 and 2020. Cultivar Halisbey, Virginia market type of peanut, was used as a plant material. The MF, consisting of *Chlorella* spp (1×106 kob mL^{-1}), was the product purchased from a company which selling the fertilizer commercially. Chlorella algea includes the eleven amino acids (lysin, methionine, cystin, trytophan, histidine, isoleucin, leucin, phenylanin, vailin, arginine, biotin) and five vitamins (A, B1, B2, C, E) in its structure.

The pH of the clay soil used in the study was slightly alkaline (pH ~8.4). Lime and iron contents were mid-level while insufficient in organic matter, phosphorus, nitrogen and potassium. Total precipitation and average temperature during 2019 and 2020 growing period and long year were shown in Figure 1. The total precipitation was 193.8 mm in 2019 and 237.3 mm in 2020. Although long term (266.5 mm) was similar with 2020, it was a bit different from 2019. This difference resulted from April and May, 2020. The average temperatures in the studied years and long term had no significant differences. The average temperatures were 24.8°C, 24.8°C and 24.3°C in 2019, 2020 and long term, respectively.

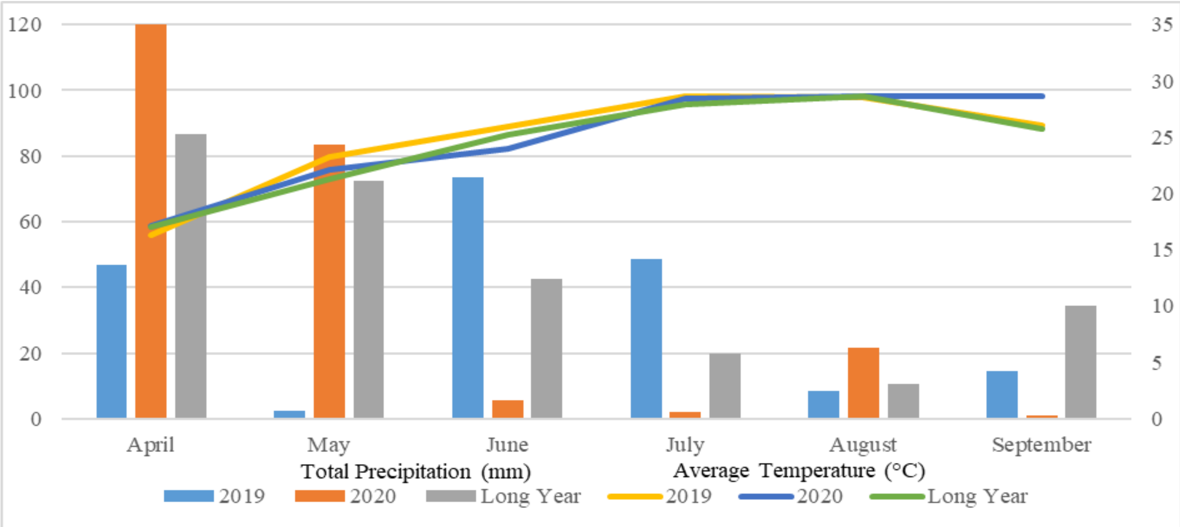

Figure 1. Meteorological data in studied years and long year average (Turkish State Meteorological Service).

Methods

Experiments were conducted in Randomized Complete Block Design (RCBD) in split-plots design with three replications. The growth stages (beginning bloom, full bloom and beginning bloom + full bloom) were placed in main plot and doses of the MF (0, 50, 100, 150 cc ha^{-1}) were placed in sub-plot. Each plot had 5 m long four rows with 70 × 15 cm spacing. Di-ammonium phosphate (18% N, 46% P_2O_5) fertilizer was used at the rates of 25 kg ha^{-1} before sowing. Sowing was performed on May 3, 2019 in the first year and on April 29, 2020 in the second year. Hand weeding was performed with the emergence of the plants. In both years of the experiment, the areas were irrigated with furrow irrigation in five times during the vegetation period of the plant. The doses of MF, given by foliar, were applied in beginning bloom (June 14, 2019; June 10, 2020) and full bloom (June 24, 2019; June 19, 2020) stages using manual sprayer machine. Manuel harvests were performed on September 25 in the first year and on September 18 in the second year. Harvests were performed from two inner rows by taking into consideration side effects.

Conventional soxhlet apparatus which consisted of a distillation flask, sample holder (thimble), siphon and condenser was used for extracting the seed oil, and solvent material was diethyl ether. The percent of peanut oil content was determined using the formula below:

$$\text{Oil content (\%)} = \frac{\text{Weight of oil extracted (g)}}{\text{Weight of the seed sample (g)}} \times 100$$

Analysis of fatty acids composition and temperature program were performed according to the method reported by Sahin et al. (2022). Iodine values (Chowdhury et al., 2015) and Oleic acid/Linoleic acid ratio were calculated with the help of the following formula:

$$\text{Iodine Values (IV)} = [(\text{oleic acid} \times 0.8601) + (\text{\% linoleic acid} \times 1.7321)]$$

$$\text{Oleic Acid/Linoleic Acid (O/L) Ratio} = \frac{\text{\% oleic acid (18:1)}}{\text{\% linoleic acid (18:2)}}$$

Experimental data were subjected to analysis of variance in accordance with split-plot design with the aid of R v4 software. Means were compared with the aid of Duncan's multiple range test (Steel and Torrie, 1980).

RESULTS

Peanut seed is one of the most remarkable protein and oil sources with its unsaturated fatty acid composition and oil content. The oil quality isn't only defined by oil content but also determined by high unsaturated fatty acid composition. The oil content was affected significantly ($p < 0.01$) by growth stages, doses of MF and stages × doses interaction in both two years according to ANOVA (Table 1). The oil contents obtained from growth stages in the present study ranged from 50.32% to 53.95%. Besides, the oil contents for doses of MF varied between 50.79-53.07%. The highest oil contents were obtained from beginning bloom + full bloom and 150 cc ha^{-1} with the value of 53.95% and 53.07%, respectively. The control groups, non-fertilizer, lower compared to MF applications for oil content in studied years.

The peanut seed is rich in monounsaturated (oleic acid) and polyunsaturated (linoleic acid) fatty acids which were affected significantly ($p < 0.01$) by growth stages and doses of MF (Table 1). Oleic acid (C18:1) varied between 48.53-50.30% for growth stages whereas it also ranged from 47.70% to 50.04% for doses. Full bloom come to forefront with the value of 50.30%. For the doses of MF, 150 cc ha^{-1} application had higher values compared to other treatments with the value of 50.04%. Linoleic acid (C18:2) ranged from 29.19% to 30.88% for growth stages, on the other hand it varied between 29.95-30.88% for doses. The highest linoleic acid contents were observed in beginning bloom (30.88%) and application of 150 cc ha^{-1} (30.88%).

The low IV and high O/L ratio provide a long shelf life and good stability for peanut. Besides, high oleic acid content of peanut oil is valuable nutrient for augmented thermos-oxidative stability and human health (Yol et al., 2017; Ergun and Zarifikhosroshahi, 2020). The ANOVA results showed a significant ($p < 0.01$) effect on O/L ratio and IV for all independent variables (Table 1). The O/L ratios varied between 1.57-1.72 for growth stages and ranged from 1.55 to 1.68 for doses. The highest O/L ratios were 1.72 for full bloom, followed by 1.58 and 1.57, beginning bloom + full bloom and beginning bloom, respectively. For doses, O/L ratio increased (1.68) till application of 150 cc ha^{-1} then decreased dramatically. The IV obtained from growth stages in the present study ranged from 93.83 to 95.22. Besides, the IV for doses of MF varied between 94.52-94.91. The lowest IVs were obtained from full bloom (93.83) and 150 cc ha^{-1} (94.91) in studied years.

Saturated fatty acids were affected significantly ($p < 0.01$) by growth stages, doses of MF and their interaction (Table 1). Palmitic acid varied between 9.32-9.64% for growth stages whereas it also ranged from 9.11% to 9.96% for doses. Beginning bloom come to forefront with the value of 9.32%. For the doses of MF, 150 cc ha^{-1} application got a better result compared to other treatments with the values of 9.11%. Stearic acid ranged from 1.91% to 2.07% for growth stages, on the other hand it varied between 1.90-2.11% for doses. The lowest stearic acid contents were observed in beginning bloom (1.91%) and control group (1.90%). As regards to arachidic acid, the values ranged from 1.96% to 2.15% for growth stages while it varied between 1.82-2.23% for doses of MF. The lowest arachidic acid was observed in full bloom (1.96%) whereas application of 100 cc ha^{-1} (1.82%) had the minimum value of arachidic acid among all treatments in studied years. Similar with results of arachidic acid, the lowest behenic acid was observed in full bloom with 2.78% and application of 100 cc ha^{-1} with 2.50%. Full bloom and application of 50 cc ha^{-1} had the minimum lignoceric acid content with the values of 1.03% and 1.01%, respectively.

As can be seen in Figure 2; oleic acid, monounsaturated fatty acid, has almost the half of total fatty acid composition, followed by one of the polyunsaturated fatty acids, linoleic acid, with the value of 30%.

DISCUSSION

Bio-fertilizers or MFs are consisted of organic matter, carriers, and beneficial microorganisms, which can improve soil fertility by maintaining the physical properties of the soil, provide nutrient absorption, and promote plant growth (Yang et al., 2020). Besides, peanut is one of the most important oil sources which has nutritious for both human and animal. There are many peanut-derived product like butter, snack etc. that are preferred by human (Sahin et al., 2022).

According to the experiment conducted by Stamford et al. (2020), MF improved the plant characteristics, promoted great effectiveness in the plant growth, and provided positive results for sustainable agriculture. Yang et al. (2020) aimed to determine the effects of MF on soil properties, microbial community structure and wheat traits. As the result of the study, MF could affect the microbial structure, soil properties and yield components of wheat positively. Besides, the change of the microbial community was correlated with growth period of wheat. Similar with these experiments, Bai et al. (2021) found that the MF with activated carbon carrier promoted alfalfa growth better than the untreated groups.

Oliviera et al. (2021) reported that MF showed significant and positive effects, as well as the best results for plant characteristics, and increased total N and available P and K compared with the control. It was also reported that the soil fertility can be improved after applying the fly ash MF and provided a green engineering approach to recycle fly ash for regreening mines (Su et al., 2021). Yan et al. (2021) found that nitrogen fixation was improved under the MF treatment, which have a significant impact for soil quality and microbial growth.

Gul and Ozturk (2021) used different MF doses to determine its effects on yield and yield components of coriander and reported that the highest oil content was recorded for the plants grown under MF treatments. Han et al. (2022) aimed to determine the optimum level of MF to promote growth of *Elymus nutans*.

Table 1. Mean of oil content, unsaturated fatty acids, saturated fatty acids, and oil quality parameters.

	Oil Content (%)	Oleic Acid (%)	Linoleic Acid (%)	O/L Ratio	Iodine Values	Palmitic Acid (%)	Stearic Acid (%)	Arachidic Acid (%)	Behenic Acid (%)	Lignoceric Acid (%)
Years (Y)										
2019	52.07±0.36	49.07±0.26 B	30.23±0.21	1.63±0.02	94.57±0.18 B	9.46±0.10 B	1.96±0.02 B	2.03±0.04 B	2.89±0.08 B	1.08±0.04 B
2020	52.00±0.42	49.17±0.26 A	30.33±0.20	1.63±0.02	94.83±0.16 A	9.52±0.09 A	2.04±0.02 A	2.12±0.04 A	2.97±0.08 A	1.23±0.03 A
Stages (S)										
BB	51.84±0.16 y	48.53±0.20 y	30.88±0.19 x	1.57±0.01 z	95.22±0.25 x	9.32±0.07 z	1.91±0.03 z	2.11±0.02 y	2.98±0.08 y	1.22±0.06 x
FB	50.32±0.32 z	50.30±0.20 x	29.19±0.13 z	1.72±0.01 x	93.83±0.09 y	9.64±0.17 x	2.01±0.03 y	1.96±0.07 z	2.78±0.11 z	1.03±0.04 y
BB+FB	53.95±0.52 x	48.53±0.38 y	30.77±0.25 y	1.58±0.03 y	95.04±0.13 x	9.51±0.07 y	2.07±0.02 x	2.15±0.03 x	3.04±0.10 x	1.21±0.03 x
Doses (D) (cc ha^{-1})										
Control	50.79±0.52 c	49.09±0.09 c	30.34±0.11 b	1.62±0.01 c	94.77±0.14 ab	9.12±0.03 c	1.90±0.04 c	2.18±0.03 b	3.16±0.02 b	1.14±0.02 b
50	51.96±0.14 b	49.66±0.15 b	29.96±0.19 c	1.66±0.02 b	94.60±0.21 bc	9.78±0.03 b	2.11±0.02 a	2.07±0.03 c	2.69±0.06 c	1.01±0.03 d
100	52.33±0.21 b	50.04±0.46 a	29.95±0.44 c	1.68±0.04 a	94.91±0.38 a	9.96±0.18 a	2.00±0.04 b	1.82±0.08 d	2.50±0.13 d	1.09±0.06 c
150	53.07±0.88 a	47.70±0.38 d	30.88±0.27 a	1.55±0.03 d	94.52±0.19 d	9.11±0.07 c	1.98±0.02 b	2.23±0.01 a	3.39±0.06 a	1.37±0.06 a
Average	52.04±0.27	49.12±0.19	30.28±0.14	1.63±0.01	94.70±9.49	9.49±0.07	2.00±0.02	2.07±0.03	2.93±0.06	1.15±0.03
Y	ns	*	ns	ns	*	**	**	**	**	**
S	**	**	**	**	**	**	**	**	**	**
D	**	**	**	**	**	**	**	**	**	**
Y×S	ns	ns	ns	ns	ns	ns	ns	ns	ns	**
Y×D	ns	ns	ns	ns	ns	ns	ns	ns	ns	**
S×D	**	ns	**	**	**	**	**	**	**	**
Y×S×D	ns	ns	ns	ns	ns	ns	ns	ns	ns	**

BB: Beginning Bloom, FB: Full Bloom, ns: non-significant. * $p < 0.05$, ** $p < 0.01$. Letters show different groups; A, B for years; x, y, z for growth stages; a, b, c, d for doses of MF.

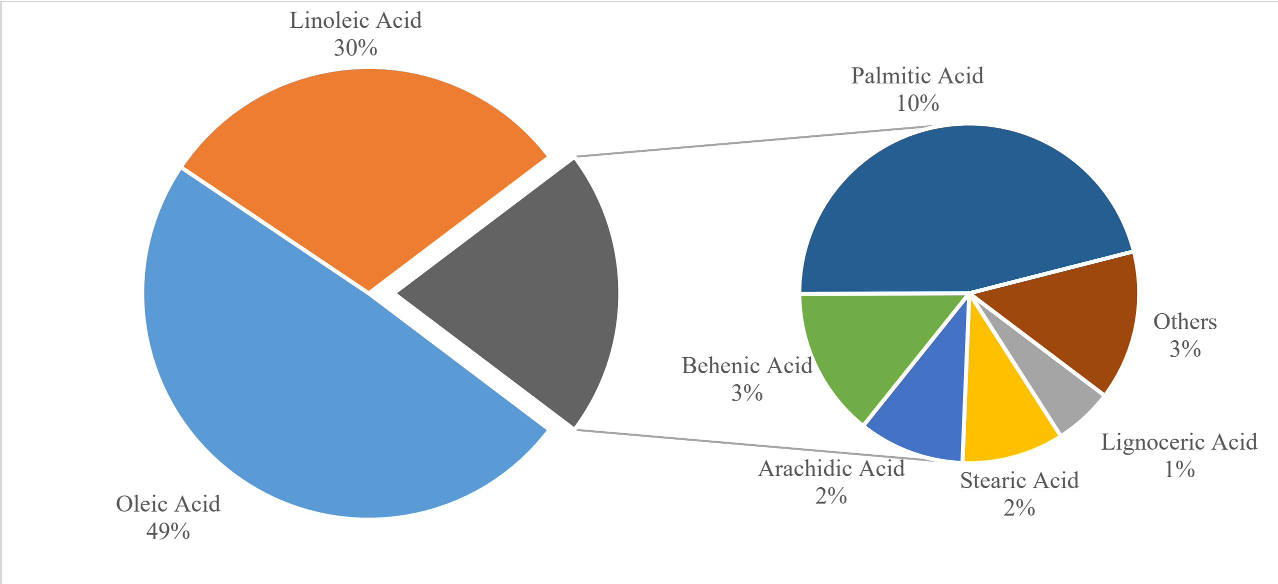

Figure 2. Fatty acid composition of peanut seed oil according to 2-year obtained data.

It was reported that MF treatments increased the oil content of plants by about 23% compared to control groups. In the current study, it was observed the increases in MF treatments which was applied in the different growth stages of peanut.

Darakeh et al. (2021) conducted an experiment with black cumin and used different types of bio-organic fertilizer (BF) or MF. They indicated that unsaturated fatty acids were affected positively by MF in comparison with untreated (control) plants. Saturated fatty acids also decreased with MF applications compared to control. Dizajeyekan et al. (2021) found that oil content and fatty acid composition of rapeseed were significantly affected by bio-fertilizer or MF treatments. The highest oil and oleic acid contents were observed in MF treatments. They recommended that bio-fertilizer or MF could improve on growth properties, oil content and fatty acid compositions. We also detected that increasing MF doses increased the unsaturated fatty acid content while decreased the saturated fatty acids in the current study.

Yol et al. (2017) and Ergun and Zarifikhosroshahi (2020) reported that high O/L ratio and low IV provide a long shelf life and good stability for peanut. High oleic acid content is valuable nutrient for augmented thermos-oxidative stability and human health. In the present study, we determined that MF applications increased the O/L ratio and decreased the IV.

The most important fatty acid compositions for peanut are oleic and linoleic acids (Table 2). There was a high negative correlation (r = -0.918) between them. It was also seen that oleic acid had significant correlation with whole fatty acid compositions. Palmitic acid had significant correlations with behenic acid (r = -0.851) and arachidic acid (r = -0.840) whereas there was a highly positive correlation between arachidic and behenic acid (r = -0.864). The oil content had a significant correlation with linoleic acid (r = 0.521) and oleic acid (r = -0.493). These results were supported by Darakeh et al. (2021) and Dizajeyekan et al. (2021). Finally, the oil content, oil quality factors and fatty acid compositions of peanut were all significantly promoted by the MF applications. Moreover, at the different growth stages applied MF affected the oil properties significantly.

Table 2. Correlation analysis for the oil content and fatty acid compositions according to average values of the studied years.

	Oil Content	Oleic Acid	Linoleic Acid	Palmitic Acid	Stearic Acid	Behenic Acid	Arachidic Acid
Oil Content	1						
Oleic Acid	**-.493**	1					
Linoleic Acid	**.521**	**-.918**	1				
Palmitic Acid	.026	**.595**	**-.552**	1			
Stearic Acid	.004	**.405**	**-.425**	**.497**	1		
Behenic Acid	**.268**	**-.822**	**.702**	**-.851**	**-.406**	1	
Arachidic Acid	.094	**-.685**	**.610**	**-.840**	-.153	**.864**	1
Lignoceric Acid	.135	**-.573**	**.404**	**-.458**	-.035	**.601**	**.630**

$p < 0.01$ in bold.

CONCLUSION

Microbial fertilizers are substance that contains microbes, which helps in promoting the growth of plants and trees by increasing the supply of essential nutrients to the plants. They are used to increase the crop yield in an eco-friendly way while relying on sustainable agriculture principles. As a result of the study, it can be said that using the MF in optimum dose and applying in time can be more profitable. Application of 100 cc ha^{-1} and full bloom may be the optimum selection.

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