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EFFECTS OF GRAFTING ON SOME PHYTOCHEMICAL TRAITS AND MINERAL CONTENT IN BITTER GOURD (*Momordica charania* L.)

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ABSTRACT

Grafting method in vegetables has been used in common due to positive effects in agriculture. In this context, it is focused that the studies aimed at increasing yield and quality through grafting. This research was carried out to determine the effects of grafting bitter gourd (*Momordica charantia* L.) onto pumpkin (*Cucurbita maxima*). Effects of grafting on extract yield, dry matter ratio, ash, and macro and micro elemental content of the fruits and leaves of bitter gourd were determined in greenhouse conditions. Grafting significantly increased the dry matter ratio and extract yield in the bitter gourd fruits and the ash content in young, mature and old leaves. In the fruits, a positive correlation (R = 0.9264) was found only between extract yield and dry matter ratio. The main important effect of grafting in terms of macro and micro elements appeared on the fruits of bitter gourd. The accumulation of Ca^{2+} in the leaves increased during maturation. The positive effect of grafting in terms of Ca^{2+} increase in fruits was higher in unripe fruits compared to ripe fruits. In addition to obtaining more extracts from the fruits bitter gourd, the enrichment of its fruits in terms of Ca^{2+} and K^+ and its leaves in terms of Ca^{2+} increases the importance of these parts in terms of human nutrition.

Key words: ash, *Cucurbitaceae*, dry matter, extract, macro and micro elements, pumpkin

INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is a popular vegetable and also used in traditional medicine in many countries. In recent years, it is one of the plants that have attracted great attention as an alternative medicine in developed countries [Raina et al. 2016]. Bitter gourd is a valuable plant adapted to in tropical and subtropical climates [Lee et al. 2015]. All parts of the plants, from root to seed, contain useful components [Duan et al. 2012]. Especially fruits and leaves of the bitter gourd are used in folk medicine in various countries such as China, India, Africa and the south-eastern US [Shubha et al. 2018]. Additionally, preclinical studies show the potential of the plant against cancer and diabetes II [Raina et al. 2016].

Preparations (capsules and tablets) produced with particular extracts from the fruit or seed of the bitter gourd are becoming increasingly common. These preparations are used for the treatment of diabetes and viral diseases including influenza and psoriasis by alternative medical practitioners [Grover and Yadav 2004]. Tsai et al. [2014] suggested that the leaves of the bitter gourd might be useful in preventing photo-oxidative damage and melanogenesis of the skin. Rachael Adebola et al. [2016] showed that antifungal compounds could be obtained from the leaves of the bitter gourd.

Kumar and Bhowmik [2010] pointed out the high levels of vitamins and minerals such as iron, calcium,

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phosphorus and vitamin B in the fruits and leaves of the plant. Hasnaa et al. [2018] reported that this plant was rich in mineral elements, especially potassium, calcium, magnesium, iron, and zinc. In addition, to these elements, phosphorus and manganese are also abundant in the plant [Shubha et al. 2018]. Therefore, the consumption of the bitter gourd contributes to the nutrient requirement of humans [Bakare et al. 2010]. Previous studies show that all parts of this plant (fruit, leaf, seed, root ect.) and their extracts are important for nutrition and pharmacy [Anilakumar et al. 2015]. Therefore, the applications that allow the increase of the bioactive compounds and some elements of this plant are important in terms of their positive effects on both human nutrition and human health.

Omar and El-hamahmy [2019] stated that the effect of grafting was positive on dry matter in the fruits and decreased or increased the amount of dry matter due to the rootstock-scion combination. Nawaz et al. [2017] and Noor et al. [2019] stated that grafting significantly increases the elements of K^+ and Ca^{2+} in cucumber fruits. On the other hand, Yetisir et al. [2013] suggested that there was no increase in K^+ and Mg^{2+} concentration in the leaves of the grafted watermelon plant, and their fruits. Fedorov et al. [2018] reported that increases or decreases in the amount of nutrients taken by plants could occur with grafting. There are also many studies showing that grafting onto various plants affects their yield and quality [Huang et al. 2009, Kowalczyk and Gajc-Wolska 2011, Moncada et al. 2013, Miskovic et al. 2016, Alzate et al. 2018, Ceylan et al. 2018].

Due to the important advantages such as resistance to diseases, tolerance to negative soil conditions, and increase in yield, grafted seedlings used in various plants are gradually increasing in the world [Bie et al. 2017]. Although there have been grafting studies on bitter gourd before, we are not aware of studies examining grafting on the bitter gourd in detail. Therefore, this study was conducted to compare grafted and non-grafted plants in terms of quality of leaves, fruits, and phytochemical traits.

MATERIAL AND METHOD

Plant material. Bitter gourd (*Momordica charantia* L.) was used as scions while pumpkin (*Cucurbita maxima*) was used as rootstocks. All seeds were sown into vials during June 2019. Emergence of scions and rootstocks seedlings was completed within 8 days. Grafting was performed 6 days after the emergence, when seedlings had 3–4 leaves. One cotyledon grafting method was used according to the procedures described by Akhila and George [2017] and Tamilselvi and Pugalendhi [2017a]. Grafted seedlings were kept in the shade for two weeks and were then transplanted into a greenhouse at a distance of 100 × 50 cm in July 2019.

Two months after planting, during the fruit development period, fruit and leaf samples were collected from five plants. Two stages of fruit maturity (unripe and ripe) and three stages of leaf maturity (young, mature, and old) were studied for some phytochemical properties. In bitter gourd, leaf maturity was classified as "young leaves" for very fresh leaves whose length is

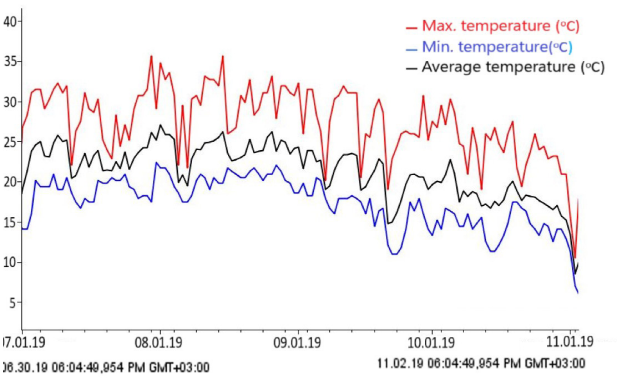

Fig. 1. Temperature values (°C) in the greenhouse

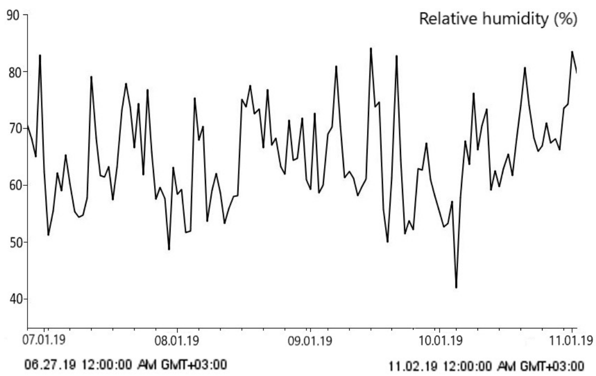

Fig. 2. Relative humidity values (%) in the greenhouse

7–10 cm, "mature leaves" for the leaves reaching yet a length of 15 cm and "old leaves" for the leaves that are 15 cm in length and lost their freshness. In pumpkin, leaf with a length of 15–20 cm was considered as young leaves, 20–25 cm as mature leaves and 25–30 cm leaves as old leaves. All of fruits and leaves were harvested simultaneously. All treatments on fruits and leaves were carried out in three replications.

Conditions of greenhouse for temperature (°C) and relative humidity (%) belonging to the period of July–October were shown in Figure 1 and Figure 2. The properties of greenhouse soil were determined in the laboratory of Atatürk Tea and Horticultural Cultures Research Institute. According to the results of analysis, the soil texture was sandy loam. Its organic matter content was 5.5%. Soil pH was 5.87 and it had high levels of nitrogen (0.28%), phosphorus (32 mg kg^{-1}) and potassium (0.42 me 100 g^{-1}). Soil salinity (1.137 dS m^{-1}) level was low and irrigation water contained very little salt (0.208 dS m^{-1}). Due to these properties of soil any fertilizer didn't applicated into the soil.

The variables evaluated were given below:

Dry matter analysis. Fresh fruits and fresh leaves collected from grafted and non-grafted plants were cut into small pieces and blended, and then kept in an oven at 105°C until a constant dry weight was achieved. Finally, ratios of dry matter to fresh weights of the were calculated in percentage.

Ash analysis. Dry plant material was weighted to 5 g per sample, kept in an ash oven at 525°C for 6 h and ash content (%) was calculated based on dry matter.

Extraction procedure. The method used by Savsatli and Seyis [2016] was modified for the preparation of extracts. 0.5 g of the dried samples were extracted at eight timepoints (ultrasonic assisted) over a period of 20 minutes with 10 ml of methanol (EMSURE, ACS, ISO, Reag. Ph Eur) in falcon tubes. After each extraction, samples were centrifuged at 4000 rpm, at 40°C for 10 minutes. Supernants of each sample were collected in a glass flask. Methanol was completely evaporated by means of the Rotary Evaporator and vacuum system. Extract yield was calculated as a percentage based on the amount of dry matter.

Elemental analysis. Fruit and leaf samples which were dried for at 105°C were used for elemental analysis. 300 mg of dried material was treated with a mixture of 5 ml nitric acid (HNO_3) (65%) and 3 ml hydrogen peroxide (H_2O_2) (30%) (Merck). After this treatment the samples were kept in a microwave decomposition device (Speedwave 4, DAK-100/4, Berghof brand). Each sample was taken into a falcon tube topped to 25 ml with ultrapure water, and kept at +4°C until analysis. Concentrations of iron (Fe^{2+}), lead (Pb^{2+}), calcium (Ca^{2+}), potassium (K^{+}), manganese (Mn^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), zinc (Zn^{2+}), aluminium (Al^{3+}) and copper (Cu^{2+}) in the materials were determined by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) and expressed in ppm. Element amounts were calculated based on the dry matter amounts of the samples.

Statistical analysis. All obtained results were analyzed in triplicate according to a randomized plot factorial experiment design by using JMP Statistical program (The Statistical Discovery Software, Version 5, SAS Institute Inc.). The data expressed in % were subjected to logarithmic transformation before statistical analyses. Tukey honest significant difference multiple comparison test was applied and the means were compared according to the significance level of 0.05.

RESULTS

Dry matter in fruit and leaf. The average dry matter ratios of the leaves of grafted and non-grafted plants with 17.77% and 17.57% were higher ($P < 0.01$) than that of *Cucurbita maxima* used as rootstock with 14.40% (Tab. 1). When a comparison is made in terms of leaf maturity stage, the dry matter ratio of young leaves was lowest with 15.37%. The values obtained from mature and old leaves were 16.87% and 17.50%, respectively (Fig. 3). The dry matter ratio in the harvested fruits of non-grafted plants was 5.47% in comparison to 5.85% of that in fruits of grafted plants. This increase was found to be statistically significant ($P < 0.01$). Ripe fruits had higher ($P < 0.01$) dry matter values than unripe fruits (Tab. 2).

Ash content in fruit and leaf. The highest average ash content was obtained from the leaves of the grafted plants with 23.65% (Tab. 1). It was followed by leaves of non-grafted plants with 20.32% and pumpkin leaves with 13.88%. These differences between the materials were found significant ($P < 0.01$). Ash content in the fruits of bitter gourd varied between 11.65% and 12.76%. Grafting had no statistically sig-

Table 1. The effect of grafting onto pumpkin on some chemical traits of the leaves of the bitter gourd

Materials (M)	Leaf maturity (L)	Dry matter (%)	Ash (%)	Extract yield (%)
Non-grafted plant	young	16.68 ±0.29	12.90 ±0.45 [f]	33.75 ±0.35 [a]
	mature	17.92 ±1.67	18.97 ±0.05 [d]	31.40 ±0.46 [ab]
	old	18.11 ±2.05	29.13 ±0.80 [b]	24.35 ±0.98 [e]
	mean	17.57 ±1.33 [a]	20.32 ±0.43 [b]	29.83 ±0.60
Rootstock (*Pumpkin*)	young	12.90 ±0.45	11.37 ±0.08 [g]	33.89 ±1.95 [a]
	mature	15.25 ±1.59	13.88 ±1.16 [f]	30.07 ±0.39 [bc]
	old	15.05 ±1.08	16.37 ±0.53 [e]	27.64 ±1.03 [cd]
	mean	14.40 ±1.04 [b]	13.88 ±0.59 [c]	30.53 ±1.12
Grafted plant	young	16.53 ±0.63	16.14 ±0.05 [e]	34.50 ±1.02 [a]
	mature	17.45 ±1.77	21.98 ±0.15 [c]	31.54 ±2.20 [ab]
	old	19.33 ±0.25	32.85 ±0.36 [a]	25.43 ±0.15 [de]
	mean	17.77 ±0.88 [a]	23.65 ±0.19 [a]	30.49 ±1.12
F values		19.9** (M) 6.7** (L) 0.7 (MxL)	760.5** (M) 1253.2** (L) 118.7** (MxL)	1.0 (M) 115.1** (L) 3.5* (MxL)
Coefficient of variation (%)		7.66	2.81	3.85

*, ** level of significance, P < 0.05*, P < 0.01**; means with the same letter are not statistically significant

Table 2. Effect of grafting onto pumpkin on some chemical traits of the fruits of bitter gourd

Materials (M)	Fruit maturity (F)	Dry matter (%)	Ash (%)	Extract yield (%)
Non-grafted plant	unripe	4.74 ±0.16	11.67 ±0.59	31.28 ±1.15
	ripe	6.20 ±0.29	12.76 ±0.88	43.61 ±1.32
	mean	5.47 ±0.23 [b]	12.22 ±0.73	37.45 ±1.24 [b]
Grafted plant	unripe	5.15 ±0.18	11.65 ±1.37	37.45 ±1.55
	ripe	6.54 ±0.09	12.19 ±0.41	50.85 ±3.11
	mean	5.85 ±0.14 [a]	11.92 ±0.89	44.15 ±2.33 [a]
F values		11.12** (M) 160.27** (F) 0.07 (MxF)	0.34 (M) 2.52 (F) 0.30 (MxF)	35.55** (M) 130.90** (F) 0.23 (MxF)
Coefficient of variation (%)		3.46	7.39	4.77

** level of significance, P < 0.01**; means with the same letter are not statistically significant

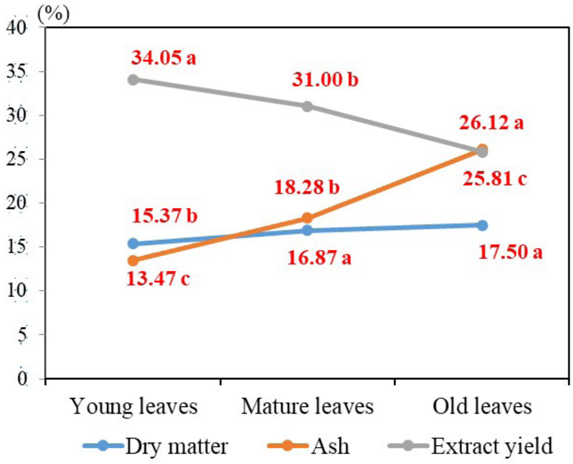

Fig. 3. General means belonging to the traits depending on leaf maturity

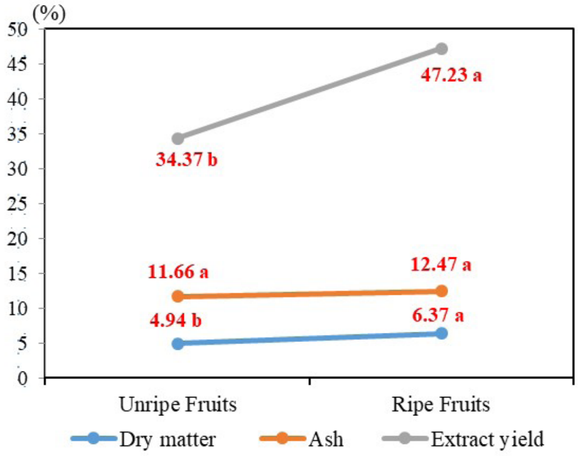

Fig. 4. General means belonging to the traits depending on fruit maturity

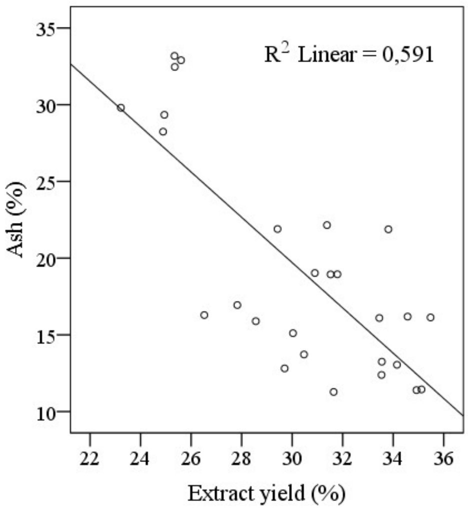

Fig. 5. Correlation between ash content and extract yield in the leaf

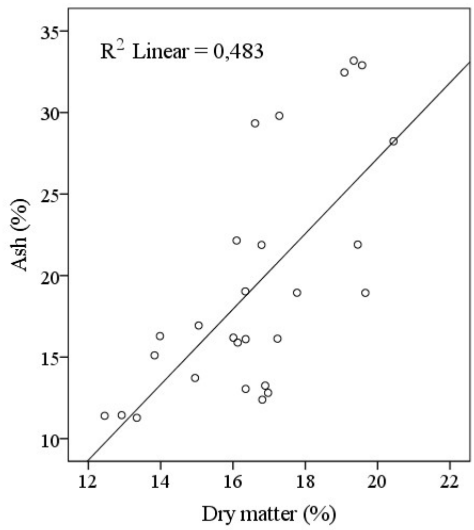

Fig. 6. Correlation between ash content and dry matter ratio in the leaf

nificant effect on the ash content of fruits. In addition, insignificant differences were found between unripe and ripe fruits in terms of ash content (Tab. 2).

Extract yield in fruit and leaf. The extract yield from leaves varied between 24.35% and 34.50% (Tab. 1). There was no statistical difference between the available materials in terms of extract yield. The extract yield of fruits ranged from 31.28% to 50.85%. Grafting significantly increased the average extract yield of fruits ($P < 0.01$) (Tab. 2). Additionally, ripe fruits had higher ($P < 0.01$) values than unripe fruits in terms of extract yield. There were significant increases ($P < 0.01$) in dry matter ratio and ash content, and significant decreases ($P < 0.01$) in extract yield as the leaves matured (Fig. 3). On the other hand, ripe fruits had higher values in terms of extract yield, ash, and dry matter ratio than unripe fruits. Increases in extract yield and dry matter ratio were found to be statistically significant ($P < 0.01$) in contrast with the increase in ash content (Fig. 4).

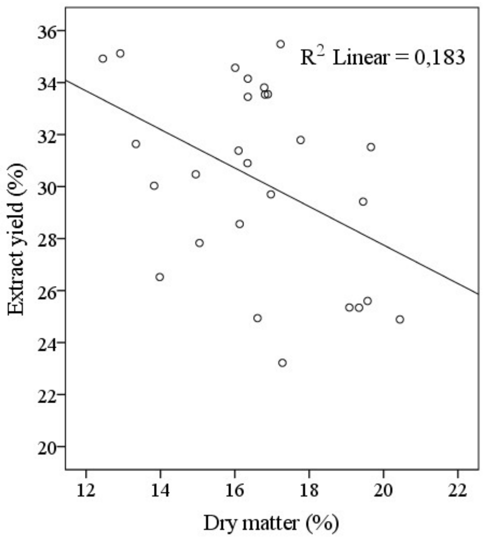

Fig. 7. Correlation between extract yield and dry matter ratio in the leaf

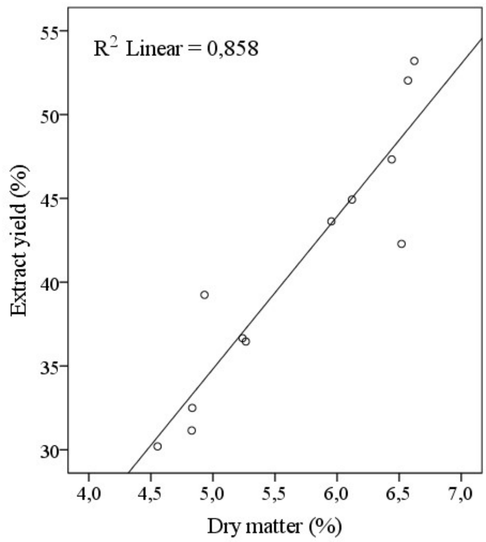

Fig. 8. Correlation between extract yield and dry matter ratio in the fruit

Table 3. Amounts of some macro elements in the dry matter (dm) of the fruits of bitter gourd grown as grafted and non-grafted (mg g^{-1} dm)

Materiale (M)	Fruit maturity (F)	Calcium	Magnesium	Potassium
non-grafted plant	unripe	2.45 ±0.37 [d]	3.10 ±0.43	39.34 ±4.13
	ripe	3.62 ±0.04 [c]	2.68 ±0.02	29.50 ±0.58
	mean	3.04 [b]	2.89	34.42 [b]
grafted plant	unripe	4.25 ±0.08 [b]	3.09 ±0.02	59.27 ±1.26
	ripe	6.02 ±0.22 [a]	2.99 ±0.15	55.32 ±2.25
	mean	5.14 [a]	3.04	57.30 [a]
F values		273.8** (M) 134.2** (F) 5.6* (MxF)	1.3 (M) 4.0 (F) 1.5 (MxF)	261.2**(M) 23.7**(F) 4.3 (MxF)
Coefficient of variation (%)		5.4	7.7	5.3

*, ** level of significance, P < 0.05*, P < 0.01**; means with the same letter are not statistically significant

Relationships among dry matter, ash content and extract yield. As a result of the correlation analysis, there was a significant (P < 0.01) and negative relationship (R = −0.7687) between ash content and extract yield (Fig. 5). While determining a significant (P < 0.01) and positive relationship (R = 0.6950) between ash content and dry matter ratio (Fig. 6), significant (P < 0.05) and negative relationship (R = −0.4273) was found between the dry matter ratio and extract yield (Fig. 7). When the same traits were taken into account for the correlation analysis in fruit, only a significant relationship (P < 0.01) was determined between extract yield and dry matter ratio. This relationship was found to be positive (R = 0.9264) (Fig. 8).

Macro and micro elements in fruit and leaf. Grafting of bitter gourd onto pumpkin, caused large

Table 4. Amounts of some macro elements in the dry matter (dm) of the leaves belonging to the pumpkin grown as rootstock with grafted and non-grafted bitter gourd (mg g^{-1} dm)

Materials (M)	Leaf maturity (F)	Calcium	Magnesium	Potassium
Non-grafted plant	young	22.40 ±0.13 [de]	5.75 ±0.02 [d]	24.37 ±0.44 [b]
	mature	49.73 ±0.53 [c]	9.06 ±0.24 [c]	14.80 ±1.60 [d]
	old	79.32 ±1.54 [b]	14.05 ±0.03 [a]	17.15 ±0.74 [cd]
	mean	50.48 [b]	9.62 [a]	18.77 [b]
Rootstock (Pumpkin)	young	12.29 ±0.08 [e]	3.87 ±0.04 [e]	24.93 ±0.89 [ab]
	mature	27.50 ±1.74 [d]	5.88 ±0.15 [d]	18.23 ±0.09 [c]
	old	28.78 ±0.57 [d]	5.68 ±0.04 [d]	27.98 ±1.65 [a]
	mean	22.86 [c]	5.14 [c]	23.71 [a]
Grafted plant	young	37.13 ±0.95 [cd]	5.56 ±0.19 [d]	24.23 ±1.01 [b]
	mature	79.45 ±14.97 [b]	8.91 ±1.47 [c]	16.00 ±1.63 [cd]
	old	96.47 ±4.48 [a]	10.69 ±0.02 [b]	14.68 ±1.00 [d]
	mean	71.02 [a]	8.39 [b]	18.30 [b]
F values		188.5** (M) 162.0** (L) 17.3** (MxL)	191.4** (M) 232.4** (L) 35.8** (MxL)	63.5** (M) 118.4** (L) 30.9** (MxL)
Coefficient of variation (%)		11.0	6.5	5.6

** level of significance, P < 0.01**; means with the same letter are not statistically significant

Table 5. Amounts of some micro elements in the dry matter (dm) of the fruits of bitter gourd grown as grafted and non--grafted (µg g^{-1} dm)

Materials (M)	Fruit maturity (F)	Copper	Aluminium	Iron	Sodium	Zinc	Manganese	Lead
Non-grafted plant	unripe	4.04 ±0.96	4.17 ±0.75	32.29 ±5.04	384.00 ±65.00 [b]	18.88 ±2.88	14.75 ±2.33 [a]	1.000 ±0.375
	ripe	5.00 ±0.67	6.29 ±1.04	32.38 ±4.21	98.96 ±2.54 [c]	14.25 ±0.50	8.08 ±0.25 [c]	0.875 ±0.333
	mean	4.52 [a]	5.23 [b]	32.34 [a]	241.48 [b]	16.57 [a]	11.42	0.938
Grafted plant	unripe	3.17 ±0.50	6.92 ±0.84	23.79 ±2.21	830.08 ±7.50 [a]	15.21 ±0.46	12.46 ±0.21 [ab]	0.792 ±0.417
	ripe	3.96 ±0.13	11.21 ±1.21	29.46 ±2.54	133.84 ±4.84 [c]	13.21 ±0.38	9.34 ±0.59 [bc]	0.709 ±0.417
	mean	3.57 [b]	9.07 [a]	26.63 [b]	481.96 [a]	14.21 [b]	10.90	0.751
F values		6.7* (M) 5.6* (F) 0.06 (MxF)	46.3** (M) 32.4** (F) 3.7 (MxF)	7.2* (M) 1.8 (F) 1.7 (MxF)	161.0** (M) 670.1** (F) 117.7** (MxF)	7.5* (M) 14.9** (F) 2.3 (MxF)	0.6 (M) 49.9** (F) 6.4* (MxF)	0.7 (M) 0.2 (F) 0.0 (MxF)
Coefficient of variation (%)		15.3	13.6	12.5	9.1	9.7	10.9	45.8

*, ** level of significance, P < 0.05*, P < 0.01**; means with the same letter are not statistically significant

Table 6. Amounts of some macro and micro elements in the dry matter (dm) of the leaves belonging to the pumpkin grown as rootstock with grafted and non-grafted bitter gourd (µg g^{-1} dm)

Materials (M)	Leaf maturity (F)	Copper	Aluminium	Iron	Sodium	Zinc	Manganese	Lead
Non--grafted plant	young	6.04 ±0.21 ^{d}	65.25 ±5.25 ^{e}	105.38 ±1.96 ^{d}	173.13 ±46.55 ^{c}	27.84 ±0.09 ^{c}	49.75 ±0.25 ^{e}	0.792 ±0.250
	mature	6.17 ±0.09 ^{d}	125.86 ±5.38 ^{de}	143.46 ±7.71 ^{cd}	551.96 ±17.29 ^{b}	26.34 ±0.34 ^{cd}	112.05 ±0.13 ^{b}	0.750 ±0.125
	old	5.92 ±0.17 ^{de}	193.75 ±14.67 ^{cd}	167.88 ±10.88 ^{cd}	885.08 ±102.50 ^{a}	44.46 ±2.38 ^{a}	197.96 ±1.96 ^{a}	1.042 ±0.000
	Mean	6.04 ^{b}	128.29 ^{b}	138.91 ^{b}	536.72 ^{a}	32.88 ^{b}	119.92 ^{a}	0.861 ^{a}
Rootstock (Pumpkin)	young	12.05 ±0.13 ^{a}	483.55 ±68.88 ^{b}	388.71 ±59.71 ^{b}	88.66 ±1.02 ^{c}	42.67 ±0.34 ^{a}	38.13 ±1.05 e	0.417 ±0.042
	mature	10.50 ±0.25 ^{b}	977.08 ±13.75 ^{a}	698.00 ±20.25 ^{a}	113.79 ±0.88 ^{c}	32.50 ±1.50 ^{b}	48.88 ±2.21 e	0.042 ±0.000
	old	10.13 ±0.30 ^{b}	466.50 ±9.17 ^{b}	361.67 ±18.84 ^{b}	124.00 ±1.51 ^{c}	32.55 ±0.38 ^{b}	42.38 ±0.95 e	0.334 ±0.209
	mean	10.89 ^{a}	642.38 ^{a}	482.79 ^{a}	108.82 ^{b}	35.91 ^{a}	43.13 ^{c}	0.264 ^{b}
Grafted plant	young	7.38 ±0.21 ^{c}	88.08 ±1.25 ^{e}	114.46 ±3.63 cd	95.10 ±0.99 ^{c}	22.79 ±1.54 ^{d}	48.04 ±0.71 ^{e}	0.417 ±0.209
	mature	4.84 ±1.09 ^{ef}	173.79 ±13.04 ^{cd}	138.30 ±18.63 ^{cd}	110.92 ±2.34 ^{c}	14.92 ±1.91 ^{e}	82.34 ±13.59 ^{d}	0.250 ±0.208
	old	3.85 ±0.16 ^{f}	205.61 ±20.86 ^{c}	180.02 ±7.69 ^{c}	124.71 ±5.63 ^{c}	13.14 ±0.94 ^{e}	98.28 ±3.45 ^{c}	0.336 ±0.207
	mean	5.36 ^{c}	155.83 ^{b}	144.26 ^{b}	110.24 ^{b}	16.95 ^{c}	76.22 ^{b}	0.334 ^{b}
F values		496.3** (M) 49.8** (L) 14.8** (MxL)	1149.1** (M) 160.5** (L) 130.3** (MxL)	637.6** (M) 67.2** (L) 67.7** (MxL)	378.6** (M) 104.6** (L) 79.9** (MxL)	553.1** (M) 65.2** (L) 111.2** (MxL)	578.6** (M) 445.6** (L) 181.9** (MxL)	34.2** (M) 4.7* (L) 1.4 (MxL)
Coefficient of variation (%)		5.5	8.3	9.2	15.0	4.5	6.0	34.4

*, ** level of significance, P < 0.05*, P < 0.01**; means with the same letter are not statistically significant

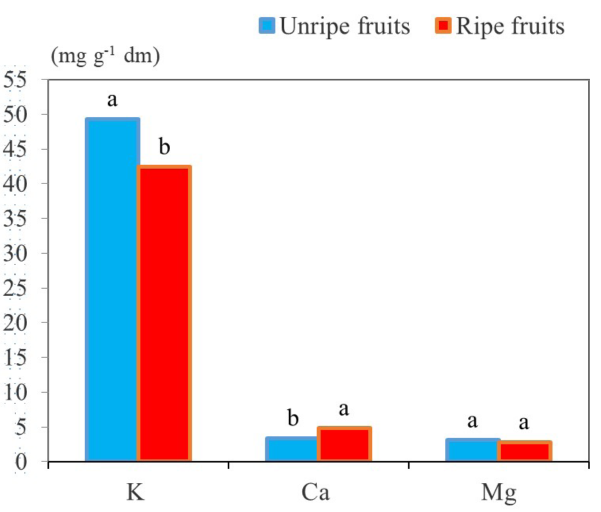

Fig. 9. Comparison of unripe and ripe fruits in terms of K^+, Ca^{2+} and Mg^{2+}

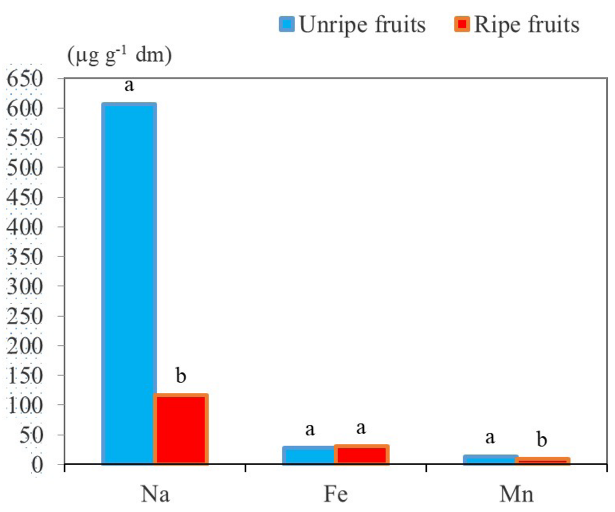

Fig. 10. Comparison of unripe and Ripe fruits in terms of Na^+, Fe^{2+} and Mn^{2+}

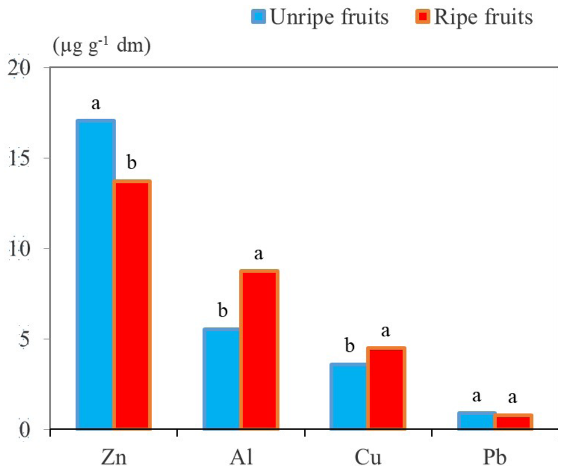

Fig. 11. Comparison of unripe and ripe fruits in terms of Zn^{2+}, Al^{3+}, Cu^{2+} and Pb^{2+}

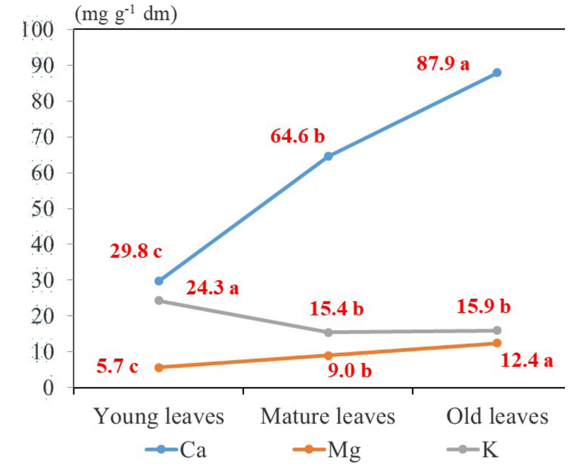

Fig. 12. Average amounts of Ca^{2+}, Mg^{2+} and K^+ in the leaves of bitter gourd

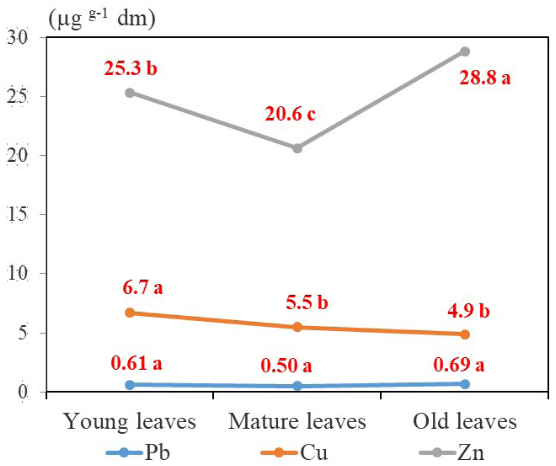

Fig. 13. Average amounts of Pb^{2+}, Cu^{2+} and Zn^{2+} in the leaves of bitter gourd

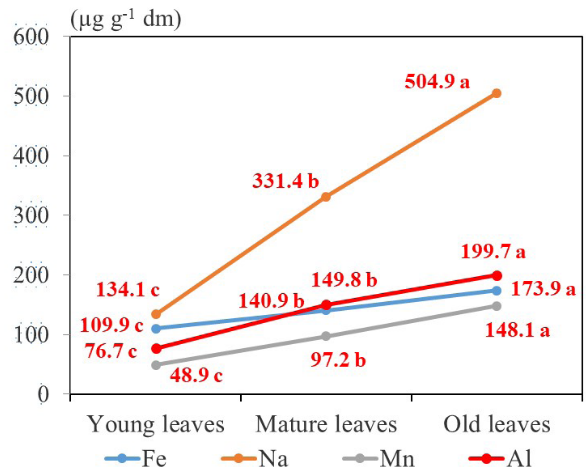

Fig. 14. Average amounts of Fe^{2+}, Na^+, Mn^{2+} and Al^{3+} in the leaves of bitter gourd

increases ($P < 0.01$) in amounts of some elements in the fruits of bitter gourd (Tabs 3, 5). The elements of Cu^{2+}, Fe^{2+} and Zn^{2+} present in the fruits of the grafted plants showed a decreasing trend ($P < 0.05$) compared to the non-grafted plants (Tabs 4, 6). The decreased ratios in these elements were calculated as 21.1%, 17.7%, and 14.2% respectively. The amounts of Ca, Cu^{2+} and Al^{3+} significantly increased, while amounts of other elements (K^+, Na^+, Zn^{2+} and Mn^{2+}) significantly decreased during the maturation of fruits (Figs 9, 10, 11). Considering the effect of leaf maturity stage on elemental uptake in bitter gourd, during the maturation of leaves, increases in amounts of Ca^{2+}, Mg^{2+}, Na^+, Fe^{2+}, Mn^{2+} and Al^{3+} as well as a decrease in amount of Cu^{2+} were determined. The amounts of K^+ and Zn^{2+} decreased in mature leaves firstly and then increased in old leaves (Figs 12, 13, 14).

DISCUSSION

Dry matter in fruit and leaf. While grafting of bitter gourd onto pumpkin did not have an effect on the dry matter ratio in the leaves, it caused a significant increase in the dry matter ratio in the fruits. As Tamilselvi and Pugalendhi [2017b] reported, grafting significantly affected the growth of the plant without decrease in quality. This positive effect may be due to the strong root system and increased photosynthesis of the rootstock [Qi et al. 2006]. The positive effect of grafting on the dry matter in the fruit was similar to the findings of Omar and El-hamahmy [2019], who stated that grafting decreased or increased the amount of dry matter of cucumber fruit due to the rootstock-scion combination.

In the current study, although grafting increased dry matter ratio in fruits, the values are below the findings with 6.5−7.6% reported by Islam et al. [2011], 6.8% reported by Yuwai et al. [1991], and 6.6−6.9% reported by Kong et al. [2017]. The low values detected in the current study may have resulted from plant material differences and growing conditions.

Ash content in fruit and leaf. Grafting onto the pumpkin significantly increased the ash content (in leaves of bitter gourd. As the leaves' maturity increased, the ash contents also increased. Similar results were obtained from the study carried by Zhang et al. [2009]. On the other hand, in terms of the same trait, grafting did not cause a significant difference between the unripe and ripe fruits of the bitter gourd.

In previous studies, the variation in ash content determined in the bitter gourd fruits was reported as 7.92−8.54% by Hasnaa et al. [2018]. Yuwai et al. [1991] determined the amount of ash in young fruits as 8.12%. In addition, the ash values were reported as 7.36% by Bakare et al. [2010], 11.12% by Fohs et al. [2014], 13.6% by Lee et al. [2015] and 2.31% by Lee [2016]. Some of these studies are in harmony, but values in others are lower than our values.

In the present study, the ash content in the leaves was much higher than the fruit. Our findings belonging to leaves were higher than the values obtained by Bakare et al. [2010] who reported an average of 15.42% and Lee et al. [2015] who reported variation between 23.2−14.4%. The highest ash content obtained from the old leaves of bitter gourd was 32.85% and is close to the extreme value 29.9% reported by Aydogan et al. [2014] who reached a high value in forage crops.

Extract yield in fruit and leaf. Grafting had no statistically significant effect on extract yield in the leaves of bitter gourd. However, extract yield of fruits increased markedly as a result of grafting. Similar to our results, Savsatli and Seyis [2016] determined the methanolic extract yields of unripe and ripe fruits of bitter gourd as 38.4% and 41.3%, respectively. Moreover, Asan Ozusaglam and Karakoca [2013] indicated that ethanolic extract yield could increase up to 63.22% in mature fruits. Used method and solvents could significantly affect extract yield depending on the plantal material.

Macro and micro elements in fruit and leaf. In the current study, the effect of grafting onto pumpkin on the amount of some nutrients in fruits and leaves of the bitter gourd was investigated. In this context, the values were calculated based on the dry matter and then given in the related tables. In some previous studies on the subject, the values obtained from the relating amount of the elements were expressed based on fresh material. For this reason, while referring to such studies, the data of current study were recalculated for 100 g of fresh material and the final values were used for the relevant literature in the discussion section.

Similar to the results reported by Lee et al. [2015], total mineral content in the leaves was higher than the fruits in our study. Obtained data from the present study showed that, as Noor et al. [2019] pointed out, the leaves of bitter gourd were especially rich in Ca^{2+}, K^+ and Mg^{2+}. When fruits and leaves are compared in terms of Ca^{2+} and K^+ content, while Ca^{2+} content is higher in the leaves, K^+ content is higher in the fruits, which is consistent with the findings reported by Lee et al. [2015]. In line with the results reported by Lee et al. [2015], elements of Mg^{2+}, Cu^{2+}, Fe^{2+}, Zn^{2+} and Mn^{2+} were higher in the leaves but Na^+ was higher in the fruits. However, the average values obtained in the study were below the maximum permissible amounts in the edible/consumable parts of the plant. Therefore, showing that bitter gourd was a useful plant as Savsatli et al. [2016] reported.

In the non-grafted plants, the amount of Na^+ was higher in leaves than fruits while it was higher in fruits in the grafted plants. With grafting, the amount of Na^+ increased in fruits while it was decreased in leaves. Moreover, it may have coincided the day when fruit and leaf samples were collected with the period when Na along with some elements was transported extensively from leaves to fruits. Colla et al. [2006]

achieved similar results in watermelon grafted onto pumpkin, in that study, amount of Na^+ decreased in leaves while increased in the fruits. This data showed that the effect of rootstock on Na^+ intake could be high level. Especially the old leaves of the non-grafted plants accumulated high levels of Na^+. The abundance uptake of Na^+ by bitter gourd shows that it is a plant to be tolerance to salt.

K^+, Cu^{2+} and Zn^{2+}, especially Fe^{2+} and Al^{3+} were found at the highest levels in the leaves of the pumpkin plant used as rootstock. Similarly, Omar and El-hamahmy [2019] reported that there were no significant differences between grafted and non-grafted plants in terms of potassium concentration in cucumber leaves. Due to the strong root system of the pumpkin plant, although it absorbed these elements from the soil intensively or its element uptake potential is high, the reflections of this on the leaves of the bitter gourd used as scion did not occured as expected. Moreover, the transport of Zn^{2+} to the leaves of bitter gourd decreased significantly ($P < 0.01$) by grafting. This result shows that only rootstock is not effective in the uptake and transportation of the elements from the soil. The response of grafted plants to the elements in soil is not only dependent on the rootstock, but also on the genotype of scion, and rootstock-scion physiological interaction [Savvas et al. 2010].

On the other hand, the most prominent effect of the scion on the fruits in terms of these elements which were uptaken by rootstock from soil intensively was observed only in K^+ and Al^{3+}. Positive effects of grafting on the K^+ and Ca^{2+} contained in the fruits of bitter gourd were in line with the findings of Nawaz et al. [2017] and Noor et al. [2019] who stated that grafting significantly increases these elements in cucumber fruits. Likewise, our data on K^+, Mg^{2+} and Ca^{2+} were similar to the results reported by Yetisir et al. [2013] who suggested that there was no increase in K^+ and Mg^{2+} concentration in the leaves of the grafted watermelon plant, and their fruits had higher values in terms of Ca^{2+} concentration than the control.

In our study, Na^+, Zn^{2+}, Mn^{2+} and Cu^{2+} decreased significantly in leaves with grafting. The tendency to decrease with grafting for Zn^{2+}, Mn^{2+} and Cu^{2+} was similar to the findings of Yetis et al. [2013], but not parallel to the results stated by Karabulut et al. [2018] who conducted a grafting study on melon. On the other hand, the lowering effect of grafting in Na^+ and Cu^{2+} content in leaves is similar to the study results of Martínez-Ballesta et al. [2010] and Huang et al. [2013].

The materials used by the researchers, environmental factors, and the methodology can normally lead to differences in trial results. Depending on the rootstock on which it was grafted, as reported by Fedorov et al. [2018], there may be an increase or decrease in the amount of elements and as reported by Noor et al. [2019], growth, yield, and fruit quality can be affected by the genotype of the rootstock. Therefore, the differences observed between the results obtained in our study and the results obtained in previous studies may have actually resulted from the differences in the sampling period [Huang et al. 2016], from the genotype structure of rootstocks [Karabulut et al. 2018] and the scion, from the rootstock-scion combination [Yetisir et al. 2013], and environmental factors.

CONCLUSIONS

Grafting of the bitter gourd onto pumpkin caused significant differences in terms of phytochemical traits in the current study. Grafting effected the elementel composition of grafted bitter gourd. The main important effect of grafting in terms of macro and micro elements appeared on the fruits of bitter gourd. The amounts of Ca^{2+}, K^+, Na^+ and Al^{3+} increased in the fruits due to grafting significantly ($P < 0.01$) while the amounts of Cu^{2+}, Fe^{2+}, and Zn^{2+} decreased significantly ($P < 0.05$).

Leaves rich in Ca^{2+} were obtained due to grafting. Further, grafting onto pumpkin also increased the dry matter ratio and extract yield in the fruits. In addition to obtaining more extracts from its fruits, the enrichment of its fruits in terms of Ca^{2+} and K^+ and its leaves in terms of Ca^{2+} increases the importance of these parts in terms of human nutrition. Therefore, it is possible to obtain more nutritious fruits and leaves from the bitter gourd grown by grafting onto *Cucurbita maxima*.

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