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ORIGINAL ARTICLE

Identification of some Fruit Characteristics in Wild Bilberry (*Vaccinium myrtillus* L.) Accessions from Eastern Anatolia

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Abstract

Some important physicochemical and bioactive characteristics of disease free 10 wild grown bilberry (*Vaccinium myrtillus*) accessions were evaluated. External and internal fruit quality was assessed by standard parameters (fruit weight, fruit color, fruit firmness, soluble solids, pH and total acidity) and bioactive contents (total phenolics, total anthocyanins, total antioxidant capacity and, vitamin C) in fruit were also determined. The commercial grown northern higbush blueberry, *Vaccinium corymbosum* cv. Bluecrop also included in the study to make comparision with bilberry samples. The highbush blueberry cv. Bluecrop had distinctive external fruit characteristics, such as bigger and more attractive fruits. However, the wild grown bilberry accessions showed interesting characters in mesocarp, such as high total phenolic content, total anthocyanin and total antioxidant capacity. Total phenolic and total anthocyanin content was 327 mg gallic acid equivalent and 142 mg of cyanidin-3-glucoside equivalent in 100 g fresh fruit in cv. Bluecrop while it was between 576–624 mg gallic acid equaivalent and 296–324 mg of cyanidin-3-glucoside equivalent in 100 g fresh fruits of bilberry accessions. Moreover, wild accessions approximately had 2 folds higher antioxidant capacity than cv. Bluecrop. Results suggested that bilberry accessions may serve as a source of desirable genes to develop improved varieties that respond to the new needs of the market.

Keywords Bilberry · phytochemicals · Genetic resources · Diversity

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Identifizierung einiger Fruchtmerkmale von Akzessionen der Wildheidelbeere (*Vaccinium myrtillus* L.) aus Ostanatolien

Zusammenfassung
Einige wichtige physikalisch-chemische und bioaktive Merkmale 10 krankheitsfreier Akzessionen wildwachsender Heidelbeeren (*Vaccinium myrtillus*) wurden beurteilt. Die externe und interne Fruchtqualität wurde anhand von Standardparametern (Fruchtgewicht, Fruchtfarbe, Fruchtfestigkeit, lösliche Feststoffe, pH und Gesamtsäuregehalt) bewertet und der bioaktive Gehalt (Gesamtphenolgehalt, Gesamtanthocyangehalt, die antioxidative Kapazität insgesamt, Vitamin C) wurde ebenfalls bestimmt. Die kommerziell angebaute Kulturheidelbeere *Vaccinium corymbosum* cv. Bluecrop wurde ebenfalls in die Studie aufgenommen, um diese mit den Proben der wilden Heidelbeere zu vergleichen. Die Kulturheidelbeere cv. Bluecrop zeigte distinktive äußere Fruchtmerkmale wie größere und attraktivere Früchte. Die Akzessionen der wilden Heidelbeere zeigten jedoch interessante Merkmale des Mesokarps, wie einen hohen Gesamtphenolgehalt, einen hohen Gesamtanthocyangehalt und eine hohe antioxidative Kapazität. Der Gesamtphenol- und -anthocyangehalt lag bei 100 g frischer Früchte der Kultursorte Bluecrop bei 327 mg Gallussäure-Äquivalent und 142 mg Cyanidin-3-Glucosid-Äquivalent, bei 100 g der Akzessionen frischer Wildheidelbeeren bei 576–624 mg Gallussäure-Äquivalent und 296–324 mg Cyanidin-3-Glucosid-Äquivalent. Zudem hatten die Akzessionen der Wildpflanzen eine etwa doppelt so hohe antioxidative Kapazität wie die Kultursorte Bluecrop. Die Ergebnisse legen nahe, dass Wildheidelbeeren als Quelle erwünschter Gene zur Entwicklung verbesserter Sorten dienen können, die neuen Anforderungen des Marktes entsprechen.

Schlüsselwörter Heidelbeere · Phytochemikalien · Genressourcen · Diversität

Introduction

Blueberries belong to the family Ericacea, genus *Vaccinium* which includes over 200 species of evergreen and deciduous woody plants ranging in size from dwarf shrubs to trees (Valentova et al. 2006; Mikulic-Petkovsek et al. 2015; Zorenc et al. 2016). The lowbush (*Vaccinium angustifolium*), the highbush (*Vaccinium corymbosum*) and the rabbiteye (*Vaccinium ashei*) blueberries along with European blueberry, with the common name bilberry (*Vaccinium myrtillus*), are the major species of *Vaccinium spp.* (Riihinen et al. 2008).

Bilberry, also known as blaeberry, heidelberry, huckleberry, hurtleberry and whortleberry, is a low-growing shrubby perennial plant that is native to northern and central Europe as well as North America, commonly spreading on the herbaceous layer of boreal forests (Valentova et al. 2006; Matsunaga et al. 2010). Because of the fact that the cultivation of bilberry is extremely difficult, it is cultivated on a very limited area. Fruits are mainly picked up from the wild plants spread on the forests and meadows of Finland, Sweden, Norway, Alpine countries, Belarus, Bulgaria, Poland and northern parts of Turkey and Russia (Jaakola et al. 2001). Bilberry is mainly cultivated in North America. France, The Netherlands, Germany, Poland and Spain are leading producers among European countries (Diban et al. 2007). However, there is an increasing interest for cultivation and breeding of bilberry because of its high nutritive value related to appreciable levels of anthocyanins, vitamins, sugar and pectins (Jaakola et al. 2001; Mauray et al. 2010).

The fruit of *Vaccinium myrtillus* is blue-black or purple in colour and differs from the American blueberry with its flesh which is purple rather than cream or white (Valentova et al. 2006). Berries of *Vaccinium myrtillus* are rich in phenolics, obvious of their deep coloration (Kähkonen et al. 2003; Riihinen et al. 2008; Cocetta et al. 2012). Bilberry is one of the most dignified wild berries with several beneficial effects in human health and nourishment (Kalt and Dufour 1997; Zhang et al. 2004). *V. myrtillus*, with long medical history dating back to the Middle Ages, is recommended especially for improving night vision, and in treating ocular disorders; it is also useful in the treatments of inflammatory conditions of the joints and peripheral vascular diseases (Pizzorno and Murray 1987). Fruit of bilberry is not only consuming fresh, but also processed into preserves, juice, wines, liquors and extracts (Primetta et al. 2012).

Together with growing popularity of wild berry consumption, cultivation area of berries broadens in consequence of breeding programs (Koca and Karadeniz 2009). Pomological features of berries are highly influenced by the species and variety within species and the ecological conditions of the plants (Mikulic-Petkovsek et al. 2012, 2014). Despite a great range of researches conducted comprehensive studies on fruit properties of blueberries (Smolarz 2006; Beccaro et al. 2006; Celik 2009; Giovanelli and Buratti 2009; Zorenc et al. 2016), the knowledge available on bilberries native to Turkey is scarce. Turkben et al. (2008) determined some pomological properties viz., fruit weight, color, as well as pH, titrable acidity and soluble solid content of a few wild bilberry fruits grown in western part of Turkey.

To our best knowledge, this is the first study conducted on fruit characteristics of wild bilberry population native to eastern Anatolia in Turkey. Hence, the objective of this investigation was to obtain an idea about fruit characteristics of wild bilberry population grown naturally in Ardahan province located in eastern Anatolia of Turkey.

Materials and Methods

Plant Material

In the current study, ripe berries of wild *V. myrtillus*, grow naturally in Ardahan province located at latitude, longitude and altitude of 41° 16′ N, 42° 70′ E and 1900 meters above sea level, respectively were sampled. The common blueberry cultivar Bluecrop also included in the study. Berries were sampled at dark-blue color in mid-August, 2014 and 2015 years from 10 pre-selected bilberry accessions that has better horticultural characteristics (high yield, free pest and diseases, having bigger fruits).

Determination of Phtysicochemical Fruit Characteristics

Fruit weight, external fruit color (L, a, b values), titrable acidity and soluble solid content (SSC) were determined on a total 50 fruits per accession in respect to five replications, with ten berries in each replication. Fruit weight values of each fruit were measured with an electronic balance of 0.01 g sensitivity. Skin color of fruits was measured with a Minolta Chroma Meter CR-400 (Minolta-Konica, Japan). Chroma meter was calibrated to a standard white reflective plate and used Commission Internationale de l'Eclairage (CIE) illuminant C. L* (lightness), a* (green to red) and b* (blue to yellow) values were measured. Colour readings were taken four times at the equatorial region of each fruit and averaged to give a mean value for each fruit (Bernalte et al. 2003). Fruit firmness was measured with a non-destrictive computerized device. The firmness for 50 randomly selected berries from each replicate was expressed as a gram-force causing fruit surface to bend 1 mm. The fruits were peeled manually and cut into small pieces and juiced. The soluble solid content (SSC) was measured in the filtered juice using a digital refractometer. The juice was also analyzed for pH and titratable acidity, which determined following the guidelines of the official AOAC method (AOAC 1995).

Determination of Bioctive Contents

Sample Preparation and Extraction Procedure

For the total phenolic, total anthocyanins and total antioxidant capacity analyses, harvested berry samples were frozen and stored at –20 °C until analysis. After thawing to room temperature, triplicate of 100 g lots of bilberry and blueberry berries from each accessions/cultivar were homogenized in a blender and they were screened for their total phenolic, total anthocyanin and antioxidant capacity following a single extraction procedure (Singleton and Rossi 1965). For this procedure, 3 g aliquots of each homogenate were transferred to polypropylene tubes and extracted with 20 mL of extraction buffer containing acetone, deionized water, and acetic acid (70:29.5:0.5 v/v), for one hour.

Total Phenolic Content

Total phenolic contents were measured according to Singleton and Rossi (1965). To determine the levels of total phenolics, 1 mL of each extract was combined with Folin-Ciocalteu's phenol reagent and water 1:1:20 (v/v) and incubated for eight minutes, followed by the addition of 10 mL of 7% (w/v) sodium carbonate. After two hours, the absorbance of each was measured at 750 nm. The values of total phenolic were estimated by comparing the absorbance of each with those of a standard response curve generated with gallic acid. Results were expressed as µg gallic acid equivalents on a fresh weight (FW) basis (mg GAE per 100 g fresh weight).

Total Anthocyanin Content

Total anthocyanin content were estimated by a pH differential method (Giusti and Wrolstad 2001) using a UV-vis spectrophotometer. Absorbance was measured at 533 and 700 nm in buffers at pH 1.0 and 4.5 using A = (A535–A700)pH1.0 (A535–A700)pH4.5 with a molar extinction coefficient of 29,600. Results were expressed as mg of cyanidin-3-glucoside equivalent in 100 g fresh weight basis.

Determination of Fruit Antioxidant Capacity

Antioxidant capacity was determined with FRAP (Ferric reducing antioxidant power) assay. In FRAP assay, 2.95 mL aliquot of a FRAP reagent, a mixture of 0.1 mol/L acetate buffer, 10 mmol/L TPTZ, and 20 mmol/L ferric chloride (10:1:1 v/v/v), were combined with 50 µL of acetone fruit extract. To determine the antioxidant capacity of the samples, the absorbance values were compared with those obtained from the standard curves of trolox (10–100 µmol/L).

Table 1 Physicochemical characteristics of bilberry accessions and blueberry cv. Bluecrop (mean of 2014–2015)

Accessions	Fruit weight (g)	Fruit external color			Fruit firmnesG/mm	SSC (%)	pH	T. acidity (%)
		L	a	b				
AR-1	0.32bc	18.3ab	0.60^{NS}	1.44ab	90b	12.1bc	3.06ab	1.08cd
AR-2	0.24c	17.3ab	0.65	1.51ab	77b	11.76c	3.04ab	1.14bc
AR-3	0.28bc	16.1ab	0.58	1.19ab	87b	12.0bc	3.10a	1.05cd
AR-4	0.34bc	20.2ab	0.72	1.42ab	79b	11.8bc	3.06ab	1.10c
AR-5	0.25bc	19.1ab	0.62	1.18ab	75b	11.5c	2.90ab	1.16bc
AR-6	0.28bc	18.9ab	0.66	1.10ab	93b	12.5b	3.06ab	1.19b
AR-7	0.23c	13.9b	0.55	1.06b	88b	12.0bc	3.11a	1.10c
AR-8	0.30bc	14.7ab	0.62	1.21ab	83b	11.9bc	2.96ab	1.02cd
AR-9	0.34b	18.6ab	0.66	1.61ab	85b	11.6bc	3.00ab	1.00d
AR-10	0.32bc	15.1ab	0.70	1.34ab	88b	11.7bc	3.07ab	1.07cd
Bluecrop	0.83a	32.4a	0.68	4.71a	277a	13.3a	2.85b	1.31a

Different letters indicate the statistical difference within same column among accessions at 5% level

The antioxidant capacity values were expressed as trolox equivalent µmol per g fresh weight basis (Benzie and Strain 1996).

Vitamin C

Ascorbic acid (Vitamin C) of samples was quantified with the reflectometer set of Merck Co (Merck RQflex) and expressed mg per 100 g fresh fruit.

Statistical Analysis

The experiment was a completely randomized design with five replications. Data were subjected to analysis of variance and means were separated by Duncan multiple range test at $P < 0.05$ significant level. There was no differences between years thus the data of both years were pooled.

Results and Discussion

Physicochemical Properties

General descriptive statistics for physicochemical characteristics regarding wild bilbery and blueberry cv. Bluecrop picked from Ardahan province are given in Table 1. One-way Anova for all parameters, except b color values, shows that significant differences between samples ($p < 0.005$), and data put in evidence that wild bilberry fruits clearly differentiate from the cultivated blueberry cv. Bluecrop in the main physicochemical properties such as fruit weight, fruit color, fruit firmness, SSC, pH and acidity.

The fruit weights of the accessions ranged from 0.23 to 0.34 g, respectively. The blueberry cultivar cv. Bluecrop had 0.83 g average fruit weight indicates that cultivated fruits have much larger berries than the wild ones (Table 1). Giovanelli and Buratti (2009) observed 0.28–0.29 g fruit weight for bilberry and 0.80–1.60 g for highbush blueberry in Italy. Turkben et al. (2008) reported fruit weight between 0.20–0.27 g among 6 wild grown *V. myrtillus* samples naturally grown in western Turkey. Our results showing agreement with above literatures on fruit weight.

Fruit L, a and b color incidence values of *V. myrtillus* accessions and blueberry cv. Bluecrop are shown in Table 1. The L, a and b values were found between 13.9–20.2; 0.55–0.72 and 1.06–1.61 among wild bilberries and it was observed 32.4, 0.68 and 4.71 in blueberry cv. Bluecrop. Turkben et al. (2008) observed fruit L, a and b color values between 11.26–16.69; 1.55–4.17 and 0.34–0.93, respectively among 6 wild grown *V. myrtillus* samples naturally grown in western Turkey. The results clearly indicating that wild bilberries are darker fruits than the cultivated ones, as demonstrated by the lower L values. Our data confirm findings by other researches on bilberry and blueberry (Prior et al. 1998; Moyer et al. 2002; Giovanelli and Buratti 2009).

Fruit firmness were found between 75 and 93 G/mm among bilberry accessions indicating statistically no differences among them. However blueberry cv. Bluecrop had higher fruit firmness (277 G/mm) than all wild bilberry samples. It can be said that blueberry cv. Bluecrop surpassed the other tested bilberries statistically ($p < 0.05$) for fruit firmness. Ochmian et al. (2009) reported fruit firmness was 86 G/mm in wild bilberries grown in Poland indicating similarity with our study. Fruit firmness is important parameter for mechanical resistance as well as transport and storage suitability. Low firmness of bilberry fruits indicating softness of these perishable fruits. The damage of fruit skin is detrimental because it affects the appearance negatively that is especially important for fruits produced for fresh market purpose. Further, cracking and damage of skin exposes fruits to oxygen causing browning of anthocyanins and pathogen attacks (Chitbanchong et al. 2009).

Table 2 Bioactive characteristics of bilberry accessions and blueberry cv. Bluecrop (mean of 2014–2015)

Accessions	Total phenolics	Total anthocyanins	Vitamin C	Antioxidant activity (FRAP)
AR-1	555d	315ab	5.31d	46.1c
AR-2	611b	307ab	6.70c	54.4ab
AR-3	604bc	322ab	8.45bc	53.0b
AR-4	582ab	301ab	7.11bc	47.2bc
AR-5	594bc	318ab	8.78b	48.4bc
AR-6	600bc	320ab	7.00bc	51.2bc
AR-7	624a	315ab	6.91bc	60.1a
AR-8	608bc	324a	7.08bc	50.8bc
AR-9	619ab	296b	7.42bc	57.2bc
AR-10	590c	310ab	7.48bc	60.6a
Bluecrop	327e	142c	39.10a	26.1d

Different letters indicate the statistical difference within same column among accessions at 5% level

SSC, pH and titratable acidity values of 10 wild grown bilberries and blueberry cv. Bluecrop are shown in Table 1. The highest SSC was detected in the blueberry cv. Bluecrop as 13.3%. Among the bilberry accessions, AR-6 had the highest SSC (12.5%), followed by the accession AR-1, which contained 12.1% SSC in its fruits. The lowest content of SSC was recorded in the fruit of accession AR-5, which had 11.5% SSC (Table 1). Turkben et al. (2008) and Giovanelli and Buratti (2009) reported SSC between 9.0–11.0% and 10.8–11.1% among wild grown *V. myrtillus* samples. Ochmian et al. (2009) also reported SSC as 13.0% in wild bilberries grown in Poland indicating higher values than our samples. The results suggest a wide genetic (within and between *Vaccinium* species) even we did not find differences among years on SSC. Different growth and environment conditions such as day length, light intensity, temperature, greatly influence the SSC of bilberries as shown by Martinussen et al. (2012). Oancea et al. (2013) reported SSC between 9.2–13.7% among wild bilberries in Romania indicating similarities with our results.

pH and titratable acidity of 10 wild grown bilberry were found between 2.90–3.11 and 1.00–1.19%, respectively (Table 1). The blueberry cv. Bluecrop had pH 2.85 and titratable acidity 1.31%. Turkben et al. (2008) reported pH and titratable acidity between 2.77–2.95 and 0.90–1.23 among wild bilberries from western Turkey. Giovanelli and Buratti (2009) reported pH and titratable acidity in the range of 3.13–3.22 and 1.00–1.18% which are in accordance to the results for bilberry fruits estimated in this study. Ochmian et al. (2009) also reported titratable acidity as 1.44% in wild bilberries grown in Poland indicating higher values than our samples.

Bioactive Contents

A high variability for anthocyanins, total phenolics, vitamin C and antioxidant activity was observed among the samples (Table 2). There were statistically significant differences among bilberry genotypes and blueberry cv. Bluecrop ($p < 0.05$) as indicated in Table 2.

Total phenolic content of bilberry accessions ranged from 555 to 638 mg GAE per 100 g fresh fruit and it was determined as 327 mg GAE per 100 g fresh fruit in blueberry cv. Bluecrop that indicate lower values than all wild bilberry accessions. The highest total phenolic content was detected in the selected accession AR-7 (638 mg GAE per 100 g), followed by the accession AR-9 (619 mg GAE per 100 g). The lowest content of total phenolic was recorded in the accession AR-1, which had concentrations of 555 mg GAE per 100 g, respectively (Table 2). The differences in total phenolic amounts expressed as GAE were deemed to be caused by genetic variation as all accessions were grown under the same ecological conditions. The average amount of total phenolic content in the fruit of *V. myrtillus* has been previously reported in Italy 577–614 mg GAE per 100 g (Giovanelli and Buratti. 2009), 640 mg GAE per 100 g fresh bilberry fruit in Poland (Ochmian et al. 2009), 429–671 mg GAE per 100 g blberry fruit in Montenegro (Jovancevic et al. 2011), respectively. Oancea et al. (2013) also reported total phenol as 355 mg GAE per 100 g in bilberry and 110 mg GAE per 100 g fruit in blueberry cv. Bluecrop. Prior et al. (1998) reported concentrations of total phenolics ranging from 233 to 273 mg GAE per 100 g in blueberry (*V. corymbosum*) cultivars and 525 mg GAE per 100 g in bilberry (*V. myrtillus*) accessions. Lee et al. (2004) investigated the polyphenolic profile of wild *Vaccinium* species from the Pacific Northwest of North America, finding a great variability on total phenolics.

The amounts of total anthocyanin content in fruits of *V. myrtillus* accessions expressed as cyanidin 3-glucoside ranged from 296 to 324 mg per 100 g. Previous studies on bilberry have reported a variation of 330–344 mg per 100 g of total anthocyanin content (Giovanelli and Buratti. 2009), and 300–698 mg per 100 g (Mazza and Miniati

1993), which is in accordance with our results. Oancea et al. (2013) reported average total anthocyanin content as 273 mg expressed as cyanidin 3-glucoside per 100 g in bilberry and 99 mg per 100 g fruit in blueberry cv. Bluecrop. Jovancevic et al. (2011) reported average 360 mg total anthocyanin expressed as cyanidin 3-glucoside in bilberry samples in Montenegro. Beccaro et al. (2006) found 327 mg anthocyanin in bilberry fruits. Anthocyanins comprise the major part of the phenolic profiles in bilberry fruits (Oancea et al. 2013). Sapers et al. (1984) and Mercadante and Bobbio (2008) reported that cyanidine, delphinidin, peonidin, petunidin and malvidin are the major anthocyanins in *Vaccinium* species. The composition and concentration of these pigments in *Vaccinium* species are influenced by different conditions, *e. g.* environmental, genetic, and physiological. Anthocyanins are distributed mainly in skin of cultivated blueberries (*Vaccinium corymbosum* L.) and both in skin and flesh of wild bilberries (*Vaccinium myrtillus* L.) also known as European blueberry. The berries of *V. myrtillus* had a higher anthocyanin content than highbush blueberry, because of their smaller size (and greater skin/flesh ratio) and due to the red colour of the bilberry flesh. Anthocyanins have potential health benefits independent or additional to their antioxidant effects (Morazzoni and Bombardelli, 1996).

Wild grown bilberries showed low vitamin C content which found between 5.31 and 8.78 mg per 100 g fresh samples indicating significant statistical differences among *V. myrtillus* accessions. However blueberry cv. Bluecrop had exceptionally higher vitamin C content (39.10 mg per 100 g) compared to wild bilberries. Due to low Vitamin C content of bilberry fruits, there were a few study in literature on this pheonema. Ochmian et al. (2009) reported average 7 mg Vitamin C in 100 g bilberry fruits and 34 mg in cultivated blueberry cv. Duke in 100 g fruits in Poland. Noormets et al. (2006) reported average 6 mg Vitamin C in bilberry fruits in Estonia.

In present study, the total antioxidant capacity of bilberry samples were found between 46.1–60.6 expressed as µmol trolox equivalent per g in FRAP method. The blueberry cultivar Bluecrop had 26.1 µmol trolox equivalent per g indicating lower value than all bilberry accessions (Table 2). It appears that AR-10 and AR-7 accessions synthesize/accumulate higher levels of antioxidants in its fruits than the rest of the accessions. Giovanelli and Buratti (2009) found the iron-ion-reducing antioxidant power values in the fruits of *V. myrtillus* genotypes as 53–57 µmol trolox equivalent per g. They also reported these values as 24 µmol trolox equivalent per g in blueberry cv. Bluecrop. Beccaro et al. (2006) reported 3 times higher antioxidant activity in bilberry fruits than blueberry cultivars determined by FRAP method. To the best of our knowledge, the antioxidant activity of *V. myrtillis* has not previously been evaluated by the FRAP assay in Turkey and in literature there was a few results on this pheonema.

Previous studies conducted on different cultivars/genotypes belongs to different fruit species indicated that fruit species are genetically very diverse group considering bioactive contents and they play a major role in modern society end economy (Canan et al. 2016; Yazici and Sahin 2016).

Conclusion

Knowledge about some physical and chemical characteristics of the wild bilberry fruits is very limited in the world and especially in Turkey. This preliminary examination in the current paper is needful for physical and chemical identification on bilberry population grown naturally in Ardahan province forest, which had not been investigated previously. However, due to fact that number of studies on the pomology of bilberry is scanty in the literature, the current study was discussed with previous studies regarding blueberry which is very similar to bilberry with respect to berry characteristics.

Conflict of interest A.M. Colak, M. Kupe, M.R. Bozhuyuk, S. Ercisli and M. Gundogdu declare that they have no competing interests.

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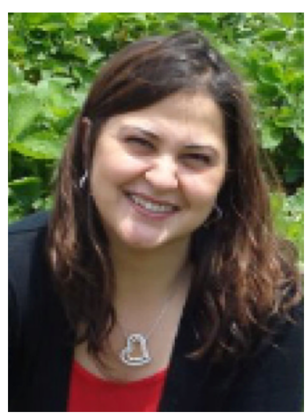

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