



Article Eco-Physiological Responses of Black Chokeberries as Affected by Applications of Oil Cake

Hyun-Sug Choi

Department of Horticulture, Daegu Catholic University, Gyeongsan-si, Gyeongbuk 38430, Korea; hchoiuark@gmail.com

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Abstract: This study was carried out to examine the optimum amount of oil cake necessary for the desired nutritional status of "Nero" black chokeberry (Aronia melanocarpa (Michx.) Elliot) in an experimental field plot between the years 2018 and 2019. The treatments included 0% (0.0 kg/ha), 25% (4.4 kg/ha), 50% (8.8 kg/ha), 75% (13.1 kg/ha), and 100% (17.5 kg/ha) of a recommended amount of oil cake. The pH in the plots with 8.8, 13.1, and 17.5 kg per ha applied ranged between 7.0 and 7.3, and these values were lower than the values observed on the plots with 0.0 and 4.4 kg per ha applied at the end of July in the years 2018 and 2019, with the concentrations of soil NO_3 -N and NH₄-N remaining low in the off-season. The foliar concentration of total-nitrogen (T-N) was higher for the plants treated with all the oil cake treatments in 2018 and with the oil cake of 17.5 kg/ha in 2019 compared to that of 0.0 kg/ha. The foliar soil plant analysis development values for June and August increased on the bushes treated with 13.1 and 17.5 kg per ha in both the years of 2018 and 2019. The cane diameter, canopy width, and total dry weight were significantly increased by bushes treated with 8.8, 13.1, and 17.5 kg per ha in both years. The fruit yield, harvest index, and percentage of T-N partitioning into fruit were maximized by the treatment with 13.1 kg per ha. An amount of 75% of the recommend application for young black chokeberry may be the prominent application rate in terms of maximized fruit productivity while balancing with the demands of vegetative growth in order to reset the recommended amount of fertilizer.

Keywords: aronia; black chokeberry; organic; partitioning; nutrient

1. Introduction

Black chokeberry (Aronia melanocarpa (Michx.) Elliot) is commonly known as an aronia, with 1.8–2.4 m heights typical of the deciduous shrub, which exhibits a dark black or purple skin color upon berry ripening [1–7]. "Viking" and "Nero" are the major cultivars of black chokeberry commercially grown in South Korea, with a monsoon climate highly weathering the moderately acidic soil [7,8]. Black chokeberry fruit contains some of the highest levels of antioxidant substrates, known to prevent modification and damage in human body cells [4,7,9,10]. However, the taste is extremely astringent due to the high tannin content, and is tart enough to cause choking in the mouth. Black chokeberry fruit is conventionally produced and consumed as a fruit powder rather than as fresh fruit, and it is traded with low fruit prices on the global market due to its year-round availability and high production yield [1,2,4,7,9,10]. Organic farming is a production system based of four main principles—health; ecology; fairness; and the restricted use of chemically synthesized substances, such as soluble fertilizers, pesticides, and herbicides [11]. Organic fruit production offers premium quality and high prices and is particularly suitable for farmers in S. Korea and other countries typically cultivating small farms of less than 1.0 ha [12]. Black chokeberry is moderately resistant to damage by insects and disease due to the highly acidic taste of the fruit, and is resistant to spring frost due to its late flowering, as well as being tolerant to drought stress [2,5–7], facilitating organic production

over any other fruit commodity. However, most black chokeberry research focuses not on the area of cultivation physiology, in particular for the function of bio-organic fertilizer, but on the health-related properties of the fruit reported in the medical references [2,5–7].

The optimum application of fertilizer achieves a balance between the annual vegetative and productive fruit yield, maintaining a sustainable orchard productivity [2,13–18]. However, the annual application of fertilizer on black chokeberry is based on the standard fertilizer program listed on the manual references of blueberry grown under acidic conditions [13,15,17,18], as little information is available on the chokeberry, which is conventionally cultivated in the western USA during the growing season [6] or in Northern Europe [2], with no information available for Korea and other countries. Pelletized oil cake is extracted from various seeds and plant residues and is widely used for the organic nutrition of fruit trees in South Korea, along with most other Asian countries [19]. Oil cake is an odorless and sterilized organic fertilizer, requiring water contents of less than 20% and a sum of T-N, P, and K contents of more than 7%, which produces a consistent nutritional value like that of chemical fertilizer compared to that of manure compost. Oil cake application typically refers to the amount of actual total-N (T-N) equivalent in the recommended soluble chemical fertilizer. These complex reasons made it difficult to determine the exact amount of oil cake necessary for the proper growth of black chokeberry, possibly inducing nutritional imbalances in the soil and bushes [14].

Approximately 80% to 90% of the raw material of oil cake is imported from the USA, China, and European countries, representing a heavy burden on organic farmers due to high oil prices. This study was initiated to evaluate the optimum amount of oil cake necessary for the desired nutritional status of one-year-old "ero" black chokeberry between the years 2018 and 2019.

2. Materials and Methods

2.1. Orchard Condition

One-year-old "Nero" black chokeberries (*Aronia melanocarpa*) with an 80 cm height and 7.0 mm cane diameter were obtained from a nursery in Gyeongsan, South Korea (35° N, 127° E), in March, 2018. The black chokeberries were planted 2 m apart with 4 m between the rows at the University Agricultural Experimental Farm Station in Gyeongsan, South Korea. The experimental plots had been previously cultivated under environmentally friendly conditions with various leafy and root vegetables five years prior to the experiment, which were then organically managed without the use of chemical soluble fertilizer, insecticide, or herbicide after planting.

Treatments were randomly assigned to three black chokeberry plots, and each treatment consisted of three plots (three replications) in a randomized complete block design. The experimental unit (data unit) was a single chokeberry in the center of the triad, and the other two bushes were guard chokeberries to prevent cross-treatment contamination.

Drip-irrigation was applied with two emitters per bush when precipitation was not received on the ground surface for 3–4 consecutive days during the growing season. Bio-degradable plastic mulch covered the soil surface to suppress the weed density. All the bushes were unpruned and maintained a natural vase-shaped shrub form for both years. The soil type between the 0 and 30 cm depth was a sandy loam, comprising 57.2% sand, 34.0% silt, and 8.9% clay. The total amount of precipitation and average temperatures were 838 mm and 22.7 °C from April to August during the growing season in 2018 [20], and 821 mm and 22.0 °C in 2019 [21], with 745 mm of total precipitation and an average temperature of 21.7 °C recorded over the last 30 years (1981–2010). The general environmental condition during the growing season was a monsoon climate that was cold and dry in spring and hot and moist in summer.

2.2. Treatments

Pelletized oil cake containing 4.6% T-N, 1.4% P, 1.0% K, and 70% organic matter, with 7.0 pH and 0.3 dS/m EC (Chamjoa, Farmhannong Co., Seoul, Korea), the typical standard nutrient status of oil cake,

was used for the trial of application rates. The defined nutrient status was the same for the oil cake used in each of the application treatments. A bush with 100% of a recommended amount (RA) received 17.5 kg of T-N per hectare (ha) based on the T-N requirement for blueberry production, with 13.1 kg of T-N for 75% RA, 8.8 kg of T-N for 50% RA, 4.4 kg of T-N for 25% RA, and 0.0 kg of T-N for 0% RA [8] as an oil cake treatment. The application of pelletized oil cake was not incorporated into the soil but scattered on the soil surface, which then irrigated around the treatment plots in April of each year.

2.3. Soil and Leaf Mineral Nutrients

Soils were randomly taken at three points at a depth of 0–30 cm at the mid-canopy of the bushes, between 30–40 cm from the main stem, with a 2 cm-diameter soil probe at the end of July in the years 2018 and 2019. Each soil sample was then taken into the lab, air-dried, and passed through a 2 mm mesh sieve for soil nutrient analysis according to the Rural Development Administration (RDA) protocols [22]. The soil pH and electrical conductivity (EC) were measured with a basis of a 1:5 (v/v) mix of soil:distilled water. The soil organic matter (OM) content was estimated by calculating the organic carbon (C) through OM oxidized by K₂Cr₂O₇ and followed by the Tyurin method. A total of 5 g of each soil sample was used for the soil T-N analysis using the Kjeldahl method, the available P₂O₅ analysis using the Lancaster method, and using exchangeable cations with 1 M of ammonium acetate (CH₃COONH₄, pH 7.0). Additional soil samples were randomly taken at a depth of 0–30 cm in the mid-canopy of the bushes in October, 2019, to determine the NO₃-N and NH₄-N status using the Brucine colorimetric method and Indophenol-Blue colorimetric method, respectively.

Forty leaves from the middle of the current-year growth on each bush were randomly sampled on 30 July 90 days after full bloom in both years, and were air-dried, weighed, and then ground in a blender with four mill blades (WDL-1, Wonder blender Co., Tokyo, Japan) for macro-nutrient analyses [22]. An amount of 0.5 g of each dried sample was digested and analyzed using the Kjeldahl method for the T-N concentration; the Vandate method for the P concentration; and atomic absorption spectrometry to measure the K, Ca, and Mg.

2.4. Bush Growth and Light Intensity

The leaf chlorophyll contents were colorimetrically estimated using the portable Soil Plant Analysis Development (SPAD) 502 m (Minolta Co., Tokyo, Japan) to measure three unfolded leaves of each bush between 13:00 and 15:00 at the end of May, June, July, and August 2018 and 2019. At the same time, the seasonal PS II activity of the leaves taken for SPAD was measured using a portable fluorometer (FluorPen P100, Photon System Inc., Drasov, Czech Republic) to estimate the CO_2 assimilation rate after dark treatment for 15 min.

The number of flowers was counted on each bush on 28 April 2019. The bush height and width were measured at the end of August 120 days after full bloom in both years, when little shoot growth was visually observed. The cane diameter was also measured on those parts of canes 5.0 cm above the ground surface using digital Vernier calipers. All the fruit was harvested on 15 August, when 70–80% of the fruit started to color [22], and the fresh weight (FW) was expressed as the fruit yield per bush, dried in a dry oven at 70 °C for 7 days, after which their dry weight (DW) was measured. Three multiple bushes from each treatment, including the root, cane, leaf, and fruit, were harvested at the beginning of September, and was separately air-dried in a dry oven at 70 °C for 7 days and then measured with an electronic scale. The harvest index (%) was expressed as the percentage of fruit DW divided by the whole bush DW.

The vegetative part (root + cane + leaf) and reproductive part (fruit) were ground separately using mill blades (WDL-1, Wonder blender Co., Tokyo, Japan) for T-N analyses for the leaf mineral

nutrients [22]. The fertilizer T-N use efficiency (FNUE) and percentage of fruit T-N recovery (PFN) were then calculated, respectively, following the formula below.

FNUE (%) = [(T-N uptake in whole bush from T-N fertilized plots – T-N uptake in whole bush from T-N unfertilized plots)/rate of fertilizer T-N applied] × 100.

PFN (%) = (T-N uptake in fruit/T-N uptake in whole bush) \times 100.

The light intensity was measured at the mid-canopy of each black chokeberry 50 cm above the ground surface using a portable light analysis system (Minolta camera Co., Ltd., Tokyo, Japan) from three equally spaced points of each bush between 14:00 and 16:00 pm in mid-August, 2019.

2.5. Data Analysis

Data analysis was performed by ANOVA using Minitab Software Version 14.1 (Minitab, Inc., State College, PA, USA). Means were separated by Duncan's multiple range test (DMRT) at $p \le 0.05$. Linear regression was also performed to determine the relationship between the oil cake treatments and the vegetative and reproductive variables in the year 2019.

3. Results and Discussion

3.1. Soil Mineral Nutrients

The 8.8, 13.1, and 17.5 kg/ha-treated plots resulted in lower soil pH ranges between 7.0 and 7.3 than those values observed for the plots with 0.0 kg/ha and 4.4 kg/ha in 2018 and 2019 (Table 1). The pelletized oil cake applied in the plots would have mineralized into soluble nutrients with the bacterial nitrification of NH₄ to NO₃ or inorganic acids such as HNO₃ or H₂SO₄ [15,19,23], but which conformed to the desired pH levels to sustain annual bush growth. The soil EC and OM were not significantly different at $p \le 0.05$ among the treatment plots in both years. The soil OM in the year 2019 increased to approximately two times higher than that of 2018 due to the maintenance of the optimum temperature and moisture contents in the rooting zone under the bio-plastic mulch during the warm and humid summer season. The concentrations of T-N, P₂O₅, and K₂O in the soil were mostly not affected by the oil cake application in both years, as the soil sampling was conducted after the bushes would have already taken up the soil mineral nutrients. The high CaO concentration in the soil in 2018 would have reduced the concentration of K₂O due to the cation competition in the soil particles [23], and this was confirmed between both cations in 2019. The MgO concentration in the soil increased in the 13.1 kg/ha-treated plots in 2019 but decreased in the 8.8 kg/ha- and 17.5 kg/ha-treated plots.

All the oil cake applications resulted in low soil NO₃-N concentrations (Figure 1A) and NH₄-N within the root zone after fruit harvest in the first two seasons (Figure 1B), reducing the nutrient surplus and contamination of water resources. However, these should be further examined for the nutrient balance, in particular for the availability of micronutrients, in the soil and leaves, as they were affected by continuous application in the long term.

3.2. Leaf Mineral Nutrients

Foliar nutrient analysis presents the nutritional status, diagnosing the deficient, optimum, and excessive rates for the trees, with little information available for black chokeberry [24]. The foliar concentration of T-N was higher for the plants treated with the oil cake in 2018 and with the oil cake of 17.5 kg/ha in 2019 compared to those of the 0.0 kg/ha group (Table 2). The foliar T-N concentration in the black chokeberry treated with 0.0 kg/ha was less than 1.8% of the minimum desired level in 2018, but was sufficient in 2019 [25], presumably due to the increase in the soil OM from the decomposable bio-mulch. The foliar concentration of P was not significantly different among all the treatments in the year 2019. The foliar K concentration in 2019 was the highest for the black chokeberry with 0.0 kg/ha applied, followed by 17.5, 4.4, 13.1, and 8.8 kg per ha. The foliar K concentration may have been

affected by a dilution effect enhancing the bush growth, with a negative correlation between high foliar K concentrations and low total DW (Table 3). The foliar K concentration significantly increased from 2018 to 2019 due to high K-availability in all the orchard soils. The foliar Ca and Mg concentrations were not significantly different among all the treatments in both years.



Figure 1. Soil concentrations of NO₃-N and NH₄-N in a "Nero" black chokeberry field as affected by five rates of application of oil cake. Different lower-case letters on each datum point for each phase indicate significant differences as determined by Duncan's multiple range test at $p \le 0.05$. Bars represent the error of the means.

Table 1. Soil chemical properties of a "Nero" black chokeberry orchard 100 d after full bloom as affected by the rates of application of oil cake.

Year/Treatment (per ha)	pH EC (1:5) (dS/1	EC	ОМ	Total T-N (%)	P ₂ O ₅ (mg/kg)	ExCation (cmolc/kg)		
		(dS/m)	/m) (mg/kg)			K ₂ O	CaO	MgO
				2018				
0.0 kg	7.8 a	0.26 a	12.7 a	0.13 a	279.4 b	0.16 a	9.4 a	1.3 a
4.4 kg	7.6 a	0.26 a	10.4 a	0.12 a	303.8 ab	0.13 a	7.0 b	1.5 a
8.8 kg	7.3 b	0.27 a	6.7 a	0.12 a	342.0 a	0.14 a	8.3 ab	1.5 a
13.1 kg	7.3 b	0.26 a	11.2 a	0.13 a	351.1 a	0.14 a	7.3 b	1.4 a
17.5 kg	7.1 b	0.26 a	8.1 a	0.16 a	351.1 a	0.16 a	7.9 b	1.3 a
				2019				
0.0 kg	7.7 a	0.23 a	20.0 a	0.09 a	256.5 a	0.41 a	4.1 a	1.2 bc
4.4 kg	7.4 b	0.21 a	22.0 a	0.10 a	293.1 a	0.40 a	4.1 a	1.3 ab
8.8 kg	7.1 c	0.21 a	24.3 a	0.08 a	323.7 a	0.27 a	4.1 a	1.1 c
13.1 kg	7.0 c	0.24 a	21.8 a	0.09 a	300.8 a	0.31 a	4.0 a	1.4 a
17.5 kg	7.0 c	0.22 a	19.9 a	0.09 a	316.0 a	0.39 a	4.1 a	1.1 c
Desired level	6.0-7.0	0.00-0.20	25-35	-	400-500	0.70 - 0.80	5.0-6.0	1.5 - 2.5

Mean values (n = 3) in each column for each year followed by the same lower-case letters were not significantly different according to Duncan's multiple range test at $p \le 0.05$. Desired levels adopted from the Rural Development Administration (RDA) [25].

Vaar/Traatmant (nar ha)	Nutrient Concentration (%)							
Tear/Treatment (per ha)	Total N	Р	К	Ca	Mg			
2018								
0.0 kg	1.4 b	0.40 a	0.8 a	2.6 a	0.9 a			
4.4 kg	1.8 a	0.20 c	0.7 a	2.3 a	1.1 a			
8.8 kg	1.9 a	0.30 ab	0.8 a	2.4 a	1.0 a			
13.1 kg	2.1 a	0.20 bc	0.9 a	2.2 a	0.9 a			
17.5 kg	1.7 a	0.40 a	0.9 a	2.4 a	1.0 a			
2019								
0.0 kg	2.2 b	0.04 a	6.2 a	2.2 a	3.2 a			
4.4 kg	2.0 b	0.04 a	4.8 ab	2.1 a	3.1 a			
8.8 kg	2.3 ab	0.04 a	3.1 c	1.8 a	2.9 a			
13.1 kg	2.4 ab	0.04 a	4.5 bc	2.0 a	3.1 a			
17.5 kg	2.6 a	0.05 a	4.9 ab	2.0 a	3.0 a			
Desired level	1.8-2.3	0.1 - 0.4	0.4-0.6	0.4 - 0.8	0.1-0.3			

Table 2. Leaf macro-nutrient concentration of a "Nero" black chokeberry 90 d after full bloom as affected by the rates of application of oil cake.

Desired levels adopted from the RDA [25]. Mean values (n = 3) in each column for each year followed by the same lower-case letters were not significantly different according to Duncan's multiple range test at $p \le 0.05$. Desired levels adopted from the RDA [25].

Table 3. Growth of a "Nero" black chokeberry as affected by five rates of application of oil cake.

Year/Treatment (per ha)	Avg. Leaf Dry wt. (g)	Bush ht. (cm)	Cane Diameter (mm)	Canopy Width (cm)	Total Dry wt. (g)	No. of Flowers
			2018			
0.0 kg	1.1 b	90.0 a	8.0 b	49.5 a	-	-
4.4 kg	1.2 b	95.7 a	10.2 a	56.0 a	-	-
8.8 kg	1.3 b	88.0 a	11.0 a	59.0 a	-	-
13.1 kg	1.7 a	93.7 a	11.0 a	61.0 a	-	-
17.5 kg	1.3 b	100.3 a	10.0 a	66.8 a	-	-
			2019			
0.0 kg	1.2 b	96.7 a	10.0 b	54.3 b	512 b	37.0 b
4.4 kg	2.5 a	105.0 a	11.9 ab	63.7 ab	1020 ab	44.3 b
8.8 kg	2.1 ab	100.7 a	12.3 a	72.8 ab	1311 a	80.0 a
13.1 kg	2.1 ab	101.3 a	12.3 a	75.7 ab	1306 a	76.0 a
17.5 kg	2.4 a	113.3 a	11.7 ab	85.7 a	1141 ab	47.0 b

Desired levels adopted from the RDA [25]. Mean values (n = 3) in each column for each year followed by the same lower-case letters were not significantly different according to Duncan's multiple range test at $p \le 0.05$.

3.3. Bush Growth

The foliar SPAD values in June and August in the years 2018 and 2019 were lower for the bushes treated with 0.0 and 4.4 kg/ha (Figure 2A,B). The T-N in bushes is a constituent of the chlorophyll molecule which is responsible for photosynthesis to absorb energy from light [16,18,26], which would have increased the SPAD of the bushes treated with 17.5 and 13.1 kg per ha, resulting in high foliar concentrations of T-N. However, the seasonal PS II activity values were not significantly different between the treatment bushes in both years (Figure 2C,D). The SPAD and PS II did not decrease from May to August in both years due to the sufficient N-availability.

The average leaf DW was significantly greater for the bushes treated with 13.1 kg/ha in the year 2018 compared to the other bushes, with lower leaf DWs observed for the bushes with 0.0 kg/ha in both years (Table 3). The bush height was not significantly different among all the treatments, but the cane diameter decreased in bushes with 0.0 kg/ha. An increase in the amount of oil cake treatment was likely to expand the bush canopy width in 2019. The bushes with 17.5 kg/ha received low light intensity into the canopy due to the increase in the shade levels (Figure 3), which would have reduced the total DW and number of flowers in 2019, as observed in blueberry grown under various shade levels [27].

The total DW was significantly increased by bushes with 8.8 kg and 13.1 kg per ha, which is similarly observed in blueberry bushes applied with soluble T-N fertilizer [18]. The total DW was positively related with the cane diameter, an estimate of the vegetative growth of the bush ($r^2 = 0.9506$; data not shown), as mostly shown for deciduous fruit trees [28]. In relation to the cane diameter and total DW, it was observed that applications with 8.8 and 13.1 kg per ha increased the number of flowers.



Figure 2. Seasonal SPAD and PS II activity of a "Nero" black chokeberry from May to August as affected by five rates of the application of oil cake. (**A**) Seasonal SPAD in 2018, (**B**) Seasonal SPAD in 2019, (**C**) PS II activity in 2018 and (**D**) PS II activity in 2019. *, *** adjacent to each datum point for each sampling time indicate significant differences, as determined by Duncan's multiple range test at $p \le 0.05$ or 0.001, respectively; ns, not significantly different.



Figure 3. Linear regression analysis of the relationship between the canopy width and light interception of a "Nero" black chokeberry in mid-July, as affected by five rates of application of oil cake. * Significantly different at $p \le 0.05$.

3.4. Fruit Productivity

Bushes that received 13.1 kg/ha resulted in the highest fruit yield, followed by 8.8, 17.5, 4.4, and 0.0 kg per ha (Figure 4A), which was similarly observed for the harvest index (Figure 4B), which was previously confirmed by the effects of different fertilizer rates on the black chokeberry [2]. The harvest index, FNUE, and PFN could be indicators for balancing vegetative growth and productive fruit [13,15,17,18,28,29]. A high FNUE was ordered by low rates of oil cake application due to the low

T-N uptake in the bushes in response to increasing the applications (Figure 5A). Bushes with 17.5 kg/ha applied resulted in an FNUE less than 30% of that of bushes with 4.4 kg/ha, potentially increasing the T-N loss into the ground water or volatilization under the alkaline soils. PFN, as a percentage of the T-N partitioning from the whole bush into the fruit, was maximized by 13.1 kg/ha, while bushes with 17.5 kg/ha had a low PFN, partially resulting from the weak salt resistance from the shallow root zone for young black chokeberry bushes in the alkaline soils (Figure 5B), as observed in the salinity damage of blueberry bushes in the acidic soils [17,18].



Figure 4. Fruit yield and harvest index of a "Nero" black chokeberry as affected by five rates of application of oil cake. Different lower-case letters on each datum point for each phase indicate significant differences, as determined by Duncan's multiple range test at $p \le 0.05$. Bars represent the error of the means.



Figure 5. Fertilizer T-N use efficiency (FNUE) and the percentage of fruit T-N partitioning (PFN) of a "Nero" black chokeberry, as affected by five rates of application of oil cake. Different lower-case letters on each datum point for each phase indicate significant differences, as determined by Duncan's multiple range test at $p \le 0.05$. Bars represent the error of the means.

Although young "Nero" black chokeberry annually requires small amounts of mineral nutrients, the vegetative and reproductive growth showed a strong relationship with the oil cake applications through the correlation analysis, except for the number of flowers and PFN (Table 4).

Table 4. Linear regression of application rates of oil cake with the vegetative and reproductive growth of a "Nero" black chokeberry.

	No. of Flowers	Total Dry wt.	Fruit Yield	Harvest Index	FNUE	PFN
Significance	ns	**	*	*	**	ns

*, ** Significantly different means among treatments at $p \le 0.05$ and 0.01, respectively. ns, not significantly different.

4. Conclusions

Oil cake is a highly soluble organic fertilizer and is frequently over-fertilized to increase the yield efficiency in organic fruit and vegetable farms [19], which influences the eco-physiological responses of black chokeberries. An amount of 75% of the recommend application for young black chokeberry maximized the fruit productivity while balancing with the demands of vegetative growth, presumably reducing the stress involved in the nutritional status of the soil and bushes, as well as providing an optimal nutrient balance. It is necessary to reset the recommended amount of fertilizer in the program listed on the manual reference of blueberry production. The savings from the purchase of oil cake fertilizer would also provide a high return on the estimated income for farmers, as well as reducing the dependency on imported fertilizer, compared to using 100% of the recommend application.

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Conflicts of Interest: The authors declare no conflict of interest.

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