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APPLICATION OF COMBINED FERTILIZERS IMPROVES BIOMASS, ESSENTIAL OIL YIELD, CHEMICAL COMPOSITIONS, AND FERULIC ACID CONTENT OF *ANGELICA SINENSIS* (OLIV.) DIELS.

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Abstract

Angelica sinensis (Oliv.) Diels, called DangGui in Chinese of the family Umbelliferae is a well-known perennial medicinal plant with versatile medicinal properties in the Chinese traditional medicine. In this research, a field experiment was carried on to understand the effect of organic and chemical fertilizers on biomass, ferulic acid content, essential oil yield, essential oil content and compositions of *A. sinensis* in Minhe county of Qinghai province of China. The treatments were: control (without fertilizer), pig manure (PM, 13.5 t ha^{-1}), sheep manure (SM, 9.8 t ha^{-1}), chemical fertilizers (CF, 60, 45 and 30 kg•ha^{-1}, nitrogen (N), phosphorus (P), kalium (K), respectively), organic fertilizer (OF, 600kg ha^{-1}), pig manure + chemical fertilizers (PM+CF, 6.75 t ha^{-1} + 30-27.5-15 kg ha^{-1} N, P, K) and sheep manure + chemical fertilizers (SM+CF, 4.9 t ha^{-1} + 30-27.5-15 kg ha^{-1} N, P, K). PM+CF treatment produced 31.4% higher crop yields and 49.4% higher essential oil yield over the control, respectively. The highest mean values of chlorophyll-a (2.07mg·g^{-1}), chlorophyll-b (0.61mg·g^{-1}) and total chlorophyll (2.68 mg·g^{-1}) were acquired under the treatment of PM+CF. The major component of essential oil of *A. sinensis* is ligustilide. The highest amount of ligustilide was acquired under the treatment of PM+CF. The ferulic acid was increased (48.85% compared with control) under the treatment of PM+CF. The P and K contents of the soil were increased under the treatment of PM+CF (around 30.13% and 25.53% higher over the control, respectively). Our studies indicated that the combined application of fertilizers improved soil nutrient, leading to increase in biomass yield and improved the quality of *A. sinensis*.

Key words: *Angelica sinensis*, Fertilizers, Essential oil, Biomass yield, Ligustilide, Ferulic acid.

Introduction

Plant secondary metabolites, as an important plant compound, are playing an essential role in medicinal or pharmaceutical applications (Wondimu *et al*., 2007, Figueiredo *et al.*, 2008). Most medicinal and aromatic plants have been widely used in disease remedy, especially in the recent decades, leading to extensive demands for folk medicine. However, most medical plants synthesize minimal contents in secondary metabolites. This prompts us to adopt some measures to enhance the contents of secondary meabolites, and on the other hand also enhance the yield of the medicinal plants in the cultivation. As an important agronomical practice, fertilization plays an essential role in the quantity and quality of plant productivity (Singh *et al*., 2007; Shen *et al*., 2017; Jhaa *et al*., 2011; Bufalo *et al*., 2015; Anwar *et al*., 2005; Bajeli *et al*., 2016; Bibiano *et al*., 2019; Onofreia *et al*., 2018; Burducea *et al*., 2018; Saha *et al*., 2019; Ya *et al*., 2021). Emerging evidences reveal that the integrated use of chemical fertilizers and organic manure produce a remarkably higher yield, higher accumulation in secondary metabolites and improve the soil characteristics compared with chemical fertilizer and organic manure alone (Pandy *et al*., 2016; Bistgani *et al*., 2018; Pandey & Patra, 2015). But these studies mainly focus on the influences of integrated chemical fertilizer and organic manure on the yield and secondary metabolites of medical plants with aerial parts (Pandy *et al*., 2016; Bistgani *et al*., 2018; Pandey & Patra, 2015), few studies specially evaluate the combined impacts of chemical fertilization and organic manure on the yield and the contents of secondary metabolites in medicinal plants with roots.

Angelica sinensis (Oliv.) Diels, called DangGui in Chinese of the family Umbelliferae, is a famous perennial medicinal plant. Traditionally, the dried roots of *A. sinensis* are prescribed for treating gynecological diseases including dysmenorrhea, premenstrual, anemia, menopausal syndromes and amenorrhea (Sarke & Nahar, 2004; Tang *et al*., 2001). Modern pharmacological action shows that it can be used for managing cancer (Su *et al*., 2013), cardiovascular disease (Tsai *et al*., 2006), Alzheimer's disease (Ho *et al*., 2009). Moreover, this medicinal plant has been used as a health care products and a dietary supplement in Asia, Europe and America (Yang *et al*., 2008). Therefore, this medicinal plant has been cultivated across the China for a long time for its numerous medicinal properties and higher demands. Ferulic acid and essential oil have been described to be important components in *A. sinensis* involving quality control (Pharmacopoeia of the People's Republic of China 2015). Previous researches have used HPLC, LC and UHPLC techniques to identify and quantity the chemical constituents in *A. sinensis,* so well as for the quality control and evaluation (Wang *et al*., 2007; Li *et al*., 2009; Wang *et al*., 2007). However, reference compounds are required in these methods to recognize the structures of unknown samples. Therefore, tandem mass spectrometry (MS/MS), plays a vital role in the chemical constituents analysis of TCM, has been integrated into LC, HPLC, UHPLC (Wei &

Huang, 2015; Gui & Zheng, 2019; Wang & Shao, 2018). The integrated UHPLC-MS/MS overcome the problems of conventional chromatographic methods, and become the new methods to recognize and quantity the chemical constituents in *A. sinensis* (Wei & Huang, 2015; Gui & Zheng, 2019; Wang & Shao, 2018). However, in previous studies the chemical constituents of *A. sinensis* from different areas have been compared using UHPLC-ESI-MS/MS and UHPLC-QTOF-MS methods (Wei & Huang, 2015; Gui & Zheng, 2019). Few studies specially used these modern methods to evaluate the impact of chemical and organic fertilization on quality for *A. sinensis* in the field. In the present study, to evaluate the impacts of chemical and organic fertilization on the quantity and quality for *A. sinensis,* a randomized complete block design experiment was performed in the field in 2019. We hypothesize that nutrients would augment the yield and improve quality of *A. sinensis*. We further hypothesize that combined fertilizers is superior to single fertilizer in term of enhancing the yield and improve quality of *A. sinensi*s in the aboveground parts as in *Thymus daenensis*, *Ocimum basilicum* and *Pelargonium graveolens*.

Materials and Methods

Field trials and transplantation: Field trials were carried out in the De Xing village, Beishan Township, Minghe County (102°47.70′E, 36°24.183′N; altitude: 2,581 m above sea level) in the year 2019. The meteorological characterization of the experimental site was 4.92°C annual mean temperature and 425 mm annual precipitations as recorded by the local weather station (Weather data AgroExpert-Adamachi). The experimental soil characteristics were listed in (Table 1).

Table 1. Chemical properties of field soil (depth of 0-30 cm), sheep manure and sheep manure.

Parameter	Field soil	Sheep manure	Pig manure
Organic carbon	0.86%	15.66%	8.7%
Total N	0.17%	0.98%	0.62%
Available N	0.0065%	0.73%	0.5%
Available P	0.0022%	0.56%	0.55%
Available K	0.0106%	0.45%	0.38%

Angelica sinensis seeds were obtained from the seed base of the Group for Medicinal Plant Resources and Vegetation Restoration of Tibetan Plateau of Northwest Institute of Plateau Biology, Chinese Academy of Sciences. A specimen with the voucher number Zhou2017115, identified by Professor Guoying Zhou, was collected from seed base at the flowering stage. Seeds were sown in 2017, according to the standardized technical specifications for *A. sinensis*. Seedlings were dug out from the soil at 16 weeks after sowing, and preserved.

Treatments details: The field experiment was carried out in a randomized block design, with one factor (fertilization) and three replications. The factor included seven treatments, which were control (without fertilizer), pig manure (PM,13.5 t ha^{-1}), sheep manure (SM, 9.8 t ha^{-1}), chemical fertilizers (CF, 60-45-30 kg ha^{-1} N, P, K, respectively), organic fertilizer (OF, 600kg ha^{-1}), pig manure + chemical fertilizers (PM+CF, 6.75 t ha^{-1}+ 30-27.5-15 kg ha^{-1} N, P, K) and sheep manure + chemical fertilizers (SM+CF, 4.9 t ha^{-1}+ 30-27.5-15 kg ha^{-1} N, P, K). $CO\text{-}(NH_2)_2$ (urea), $(Ca(H_2PO_4)_2)$ H_2O (triple superphosphate) and K_2SO_4 (potassium sulphate) were used as N, P and K, respectively. Before transplanting, the pig manure, sheep manure, organic fertilizer and chemical fertilizers were added into the soil. *A. sinensis* seedlings of uniform size were transplanted into the experimental plots on April 12th 2019. The rows were spaced at 25 cm, with 20 cm between plants, and the plot size was 13m^2 (2.6m×5m).

Pesticides were not used during the experiment, and artificial weeding was used. The roots of *A. sinensis* were harvested on 20 October 2019.

Soil sampling and soil analysis: After harvesting *A. sinensis* plants, soil samples (0-30 cm depth) were collected and homogenized from each plot in both years. Soil samples were sieved through a 2-mm mesh to remove any stones and roots, and then dried at room temperature. The air-dried soil samples were used for analysis to evaluate the chemical properties. The available N, available P and available K contents were measured using the Kjeldahl method (Bao, 2000), Mo-FB colorimetric method (Lu, 2000) and ammonium acetate flame photometer method (Lu, 2000), respectively and potassium dichromate oxidation spectrophotometric method was used to measure Soil organic carbon (Bao, 2000).

Determination of *A. sinensis* biomass: After 180 days of growth in the field (October 20 2019), roots of *A. sinensis* were harvested. From each plot 1 m^2 samples were collected. We dried the samples in the shade, and then the dried roots were weighed and biomass was calculated as gm^{-2}.

Chlorophyll content: The chlorophyll was extracted from 0.2g of fresh leaf samples in 5 ml of ethanol (95 % V/V). Chlorophyll a, chlorophyll b, total chlorophyll content in leaf tissues were calculated according to the following equation (Sartory & Grobbelaar 1984; Zhen & Ma, 2018):

$$\text{Chlorophyll-a} = (13.7\times A665 - 5.76\times A649) \times V/(1000\ M)$$
$$\text{Chlorophyll-b} = (25.8\times A649 - 7.60\times A665) \times V/(1000\ M)$$
$$\text{Total chlorophyll} = (6.10\times A665 + 20.04\times A649) \times V/(1000\ M)$$

where A665 and A649 exhibit the absorbance at 665 (chlorophyll-b) nm, 649 (chlorophyll-a) nm, M=sample weight (mg) and V = extract volume (mL).

Essential oil extraction: The dried roots of *A. sinensis* plants were crushed and passed through a 60 mesh sieve. The ground root tissue (50 g) was added to a 1-L round-bottom flask containing 500 mL of distilled water. Then, the flask was connected to a Clevenger-type apparatus, as described by the Chinese Pharmacopoeia Commission (2015). Hydrodistillation was carried out for 5 h in nine replicates (n = 9), and the essential oil content (% [v/w]) and yield (gm^{-2}) were calculated using the following equations:

Essentital oil (%) = Amount of essential oil extracted amount of ground dried root x 100%

Essential oil yield = *A. sinensis* biomass yield x Precent essential oil content

LC-MS/MS analysis: The fresh root sample of *A. sinensis* was ground with 1g liquid nitrogen and 1ml 70% methanol solution was added followed by shaking in a vortex for 3 min. and then the solution was shaken every 30 minutes, repeated for 6 times and put into at 4°C refrigerator overnight. After centrifugation at 12000 rps for 15min, 300 µl supernatant was percolated through 0.2 µm membrane and evaporated to dryness. The lyophilized samples were redissolved in 1mL of H_2O/ methanol (1:1, v/v), and the supernatant was taken after centrifugation for following analysis.

The separation was performed by an analytical column Waters ACQUITY UPLC HSS T3 with particle measure of 1.8 µm and column sizes of 100mm×3mm. We set 40°C for the column temperature during separation and (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile as the mobile phases. Separation was carried on in the following gradient conditions: 0.5 min, 10%B, 5 min, 90% B, 5.1 min, 10%B, 7 min stop, with a flow rate of 0.5 mL/min. 3 µL was the injection volume.

We acquired the mass spectra adopting a QTRAP4500 system with a Duo Spray source in positive and negative ESI mode. The best parameters for positive and negative mode were as shown below: the ion spray voltage −4,500 V (negative ion mode) and 5,500 (positive ion mode), collision gas, medium, the Turbo V spray temperature, 600°C, heater gas (Gas 2), 60 psi, nebulizer gas (Gas 1), 50 psi and the curtain gas , 20 psi. MRM (Multiple reactions monitoring) mode was used to acquire the data, and the retention time related to each MRM transition was automatically set by MassLynx software. The Peak View Software™ 2.2 (SCIEX, Foster City, CA, USA) was applied to analyze the data.

Statistical analysis

One-way ANOVA was applied to determine whether differences existed between different treatments using SPSS software (version 17). LSD (the least significant difference) test at the 5% probability level was adopted to compare the mean of the treatments. The R software was used to calculate the correlation coefficient between N, P, K and biomass yield, essential oil content, yield, ferulic acid.

Results

Soil chemical properties: The different fertilizers treatment demonstrated a favorable influence on the organic C content and available N, P and K content of the soil (Table 2). The lowest and highest amounts of organic C content were found under control (8.59 g/kg) and PM+CF treatment (11.19g/kg), respectively. However, the difference was not significantly (p>0.05). Available N content had a similar pattern as organic C. The treatment of PM, CF and PM+CF significant increased the available P content when compared with control condition (p<0.05). Higher level of available K was found in SM+CF treatment (142.33 mg/kg), and it had significant difference with control.

Correlation: Significant positive correlation were acquired between soil organic C content and biomass yield, essential oil content, yield, ferulic acid content (R^2=0.932, 0.823,0.944 and 0.903, respectively, (p<0.05)) (Table 3). Meanwhile, N were positively correlated with essential oil content and yield (R^2=0.934 and 0.759, respectively, (p<0.05)), and the content of ferulic acid was positively correlated with P and K (R^2=0.857 and 0.827, respectively, (p<0.05)).

Chlorophyll content: Among different fertilizers treatments, significant augment in chlorophyll-a content of *A. sinensis* leaf was observed in combined fertilizers (PM+CF) and SM as compared to control (Table 4). While same results were obtained in total chlorophyll and chlorophyll b content of *A. sinensis* leaf, that is, chlorophyll-b and total chlorophyll content in *A. sinensis* leaf were significant higher under combined fertilizers (PM+CF) than under control (Table 4). Chlorophyll-a, chlorophyll-b and total chlorophyll content were higher in PM+CF treatments by 24.64%, 22.95% and 24.25%, respectively, over the control. However, no significant difference was found on leaf chlorophyll b and total chlorophyll content of *A. sinensis* between other fertilizer treatment and control.

Biomass yield: As shown in Table 4, dry root yield of *A. sinensis* under different treatments ranged from 500.33 to 728.75gm$^{-2.}$ Significant increase in the dry root yield of *A. sinensis* was observed under application of combined fertilizers (PM+CF) as compared to control, which increased the yield by 31.34%. However, no significant difference was observed between other fertilizer treatments and control in terms of the dry root yield.

Essential oil content and yield and compositions

Essential oil content: Essential oil has been described to be an important quality control indicator in *A. sinensis*. Results showed that all fertilizer treatments enhanced the essential oil content of *A. sinensis,* but significant differences were found between combined fertilizers, PM, OF and control. The highest essential oil content (1.03%) was recorded under combined fertilizers (PM+CF), followed by combined fertilizers (SM+CF), which showed approximately 29.13 and 26.26 (%) increase over the control, respectively (Table 4).

Essential oil yield: Various fertilization treatments had significant influence on the EO yield of *A. sinensis*. All the fertilization treatments produced higher EO yield as compared to the control (Table 4). However, only the EO yield of *A. sinensis* under PM+CF treatment was significant higher than control, and produced the highest oil yield that was 49.45% higher over the control.

Chemical components of essential oils: The principal essential oil components of *A. sinensis* are ligustilide and senkyunolide A. Various fertilization treatments increased the content of ligustilide, senkyunolide A, butylphthalide and levistolide A when compared to control. However, the significant differences were found between combined fertilizers, PM and control in term of ligustilide content. Regarding the senkyunolide A and butylphthalide, the significant differences were found between PM+CF treatment and control. All fertilization treatments significant increased the content of levistolide A was observed when compared to control (Table 5).

Table 2. Soil chemical properties of *A. sinensis* under different fertilization system.

Treatments	Organic C (g/kg)	Available N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
Control	8.59 ± 0.76a	64.93 ± 3.24a	17.74 ± 1.35b	106.00 ± 3.06b
SM	10.39 ± 1.44a	64.93 ± 10.88a	22.28 ± 2.94ab	121.67 ± 14.34ab
PM	10.62 ± 0.74a	74.73 ± 4.9a	26.58 ± 1.92a	138.33 ± 13.19ab
OF	9.95 ± 0.087a	72.51 ± 2.68a	16.67 ± 2.47b	120.00 ± 4.16ab
CF	10.17 ± 0.13a	71.05 ± 4.9a	24.36 ± 3.81a	126.33 ± 3.67ab
SM + CF	10.16 ± 1.28a	73.50 ± 9.24a	23.14 ± 2.9ab	142.33 ± 19.75a
PM + CF	11.19 ± 0.89a	74.73 ± 5.34a	25.39 ± 0.59a	127.33 ± 12.00ab

Table 3. Correlation among organic C, N, P, K, and dry root yield and quality in *A. sinensis*.

	Organic C	N	P	K	Dry root yield	Essential oil content	Essential oil yield	Ferulic acid
Organic C	-							
N	0.651^{ns}	-						
P	0.747^{ns}	0.506^{ns}	-					
K	0.668^{ns}	0.743^{ns}	0.719^{ns}	-				
Dry root yield	0.932*	0.450^{ns}	0.663^{ns}	0.479^{ns}	-			
Essential oil content	0.823*	0.934*	0.580^{ns}	0.787*	0.685^{ns}	-		
Essential oil yield	0.944*	0.759*	0.677^{ns}	0.698	0.913*	0.921*	-	
Ferulic acid	0.903*	0.534	0.857*	0.827*	0.818*	0.727*	0.836*	-

Table 4. Effect of different fertilizers on dry root yield, essential oil yield and content and chlorophyll content of *A. sinensis*.

Treatments	Root yield (gm^{-2})	Essential oil content (%)	Oil yield (gm^{-2})	Chlorophyll-a content ($mg\ g^{-1}\ FW^{-1}$)	Chlorophyll-b content ($mg\ g^{-1}\ FW^{-1}$)	Total chlorophyll content ($mg\ g^{-1}\ FW^{-1}$)
Control	500.33 ± 19.12b	0.73 ± 0.017b	3.67 ± 0.17b	1.56 ± 0.09c	0.47 ± 0.03b	2.03 ± 0.12b
SM	652.72 ± 80.38ab	0.83 ± 0.027ab	5.42 ± 0.53ab	1.82 ± 0.08b	0.54 ± 0.03ab	2.35 ± 0.11b
PM	612.09 ± 55.83ab	0.96 ± 0.041a	5.84 ± 0.36ab	1.71 ± 0.13bc	0.50 ± 0.04b	2.21 ± 0.17b
OF	574.9 ± 88.67ab	0.94 ± 0.048a	5.40 ± 0.85b	1.75 ± 0.09bc	0.50 ± 0.03b	2.25 ± 0.12b
CF	597.48 ± 42.26ab	0.93 ± 0.035ab	5.54 ± 0.80ab	1.78 ± 0.07bc	0.53 ± 0.02b	2.31 ± 0.09b
SM+CF	611.92 ± 40.34ab	0.99 ± 0.084a	6.12 ± 0.88ab	1.76 ± 0.08bc	0.52 ± 0.03b	2.28 ± 0.083b
PM+CF	728.75 ± 46.83a	1.03 ± 0.047a	7.26 ± 0.14a	2.07 ± 0.06a	0.61 ± 0.02a	2.68 ± 0.12a

Ferulic acid content: Ferulic acid has been described to be another important quality control indicator in *A. sinensis*. The ferulic acid content of *A. sinensis* was positively affected by different fertilizer treatments except for OF treatment. All the fertilizer treatments produced significant higher ferulic acid content compared to the control (Table 5). PM + CF treatment generated the maximum ferulic acid content that was 48.85% higher over the control.

Discussion

The P and K contents of the soil increased under PM+CF treatment (around 30.13% and 25.53% higher over the control, respectively). Alike, the highest mean values of other soil fertility parameters such as organic C and N were found under PM+CF treatment, although the difference were not significant compared to control. That is, remarkably improvement of the soil fertility was found in application of combined fertilizers PM + CF, which was in conformity with the findings of previous studies (Pandey *et al*., 2016; Bistgani *et al*., 2018; Pandey and Patra 2015; Anwar *et al*., 2005). The appearance of facility exploitable water dissolvable organic carbon under integrated fertilizer treatments might be one reason for the improvement of soil fertility (Manna & Ganguly, 1997). Soil micro-organisms use the exploitable water dissolvable organic carbon as a source of energy and enhance the nutrient availability in the soil.

In this study, the higher dry root yield of *A. sinensis* was achieved under application of combined fertilizers (PM+CF). Our results were supported by previous studies which showed that the higher biomass yield was induced by the integration of organic manure and chemical fertilizers (Pandey *et al*., 2016; Bistgani *et al*., 2018; Pandey & Patra 2015; Patra *et al*., 2000). This could be explained by the following reasons: First, the higher levels of chlorophyll-a, chlorophyll-b and total chlorophyll content of *A. sinensis* under the treatment of combined fertilizers (PM+CF) resulted in higher photosynthetic activity leading to higher biomass production, which was in line with the result of *Ocimum basilicum* (Pandey & Patra, 2015). Mg and Nitrogen are important elements in the formation of chlorophyll molecules (Taiz & Zeiger, 2006). Previous study showed that organic manure contained many micronutrients such as copper magnesium, manganese, iron, zink and *et al* (Amujoyegbe *et al*., 2007; Fallah *et al*., 2018). Therefore, in this study, the higher Mg and N content in the treatment of PM+CF increased the content of chlorophyll. Second, the comprehensive nutrients provided to the plants improved the growth of plant (Edmeades, 2003). The addition of nutrients from different sources (Organic manures: organic C and micronutrients, CF: availability N, P and K) promoted the formation of soil structure, improved the physical and chemical characteristics of soil, which speeded up the growth of leaves and roots (El-ashry *et al*., 2008). However, the difference was not significant between combined fertilizer treatment (SM+CF) and control in terms of the dry root yield of *A. sinensis*. This might be caused by the fact that although the content of organic matter and N in sheep manure is high, it cannot be absorbed and used by plants directly, while C: N ratio in pig manure is lower that enhanced the nutrient availability in the soil (Hsu & Lo, 1999).

Ferulic acid and essential oil have been described to be important quality control indicators in *A. sinensis* (Mao *et al*., 2002). PM + CF treatment resulted in maximum ferulic acid content (48.85% higher over the control). Other treatments except OF treatment also showed significant variation compared to the control (Table 5). Previous study has shown that soil organic matter is an important factor affecting the ferulic acid content of *A. sinensis* (Li *et al*., 2020), while significant correlation was found between the content of organic C and the content of ferulic acid in this study. Therefore, the higher contents of ferulic acid under various fertilizer treatments were caused by the higher organic C contained in soil of different fertilizer treatments. Meanwhile, the content of organic C was highest under application of combined fertilizers (PM+CF), which resulted in the maximum ferulic acid content of *A. sinensis*.

The highest essential oil content of *A. sinensis* was found under the treatment of combined fertilizers (PM+CF), followed by the treatment of PM. Previous researches manifested that nitrogen played a vital part in photosynthetic rate and the development and differentiation of essential oil-related cells (Agena, 1994; Sangwan *et al*., 2001). In this study, Pearson's correlation coefficient analyses were carried on to ascertain the impact of nitrogen on essential oil content of *A. sinensis* (Table 3). N significantly correlated with the values of essential oil content (R^2= 0.934). Primary metabolites are the precursors of secondary metabolites. The higher nitrogen supply from pig manure and chemical fertilizers enhanced photosynthesis, which provided more precursors for the synthesis of secondary metabolites. Therefore, the higher essential oil content of *A. sinensis* was acquired under PM + CF treatment. In addition, it is reported that molybdenum, manganese, zinc and boron can promote the content of essential oil of *A. sinensis*, especially molybdenum and manganese (Wang *et al*., 2009). Organic manures are rich in nutrient composition, including micronutrients, which can increase the content of essential oil of *A. sinensis*. Our results are consistent with previous research, which showed that integrated application of organic and chemical fertilizers could prominently enhance the content of essential oil (Croteau *et al*., 1972; Bistgani *et al*., 2018).

Table 5. Effect of different fertilizers on Ferulic acid and main essential oil compositions of *A. sinensis*.

Compounds	RT	*m/z* precursor ion	*m/z* product ion	CE	Treatments						
					Control	SM	PM	OF	CF	SM+CF	PM+CF
Ferulic acid (%)	2.85	193 [M-H]$^-$	134 178	22 17	0.67±0.07b	1.26±0.14a	1.30±0.15a	0.89±0.13b	1.18±0.11a	1.28±0.084a	1.31±0.14a
Ligustilide (%)	4.29	191.1[M+H]$^+$	91 117	40 29	7.35±0.86b	8.95±0.92b	12.19±0.54a	10.98±1.58ab	12.23±0.67a	11.56±0.66a	12.58±0.42a
Senkyunolide A (%)	4.03	193.1[M+H]$^+$	137 91	17 33	0.17±0.023b	0.32±0.03b	0.25±0.017b	0.21±0.025b	0.38±0.028b	0.31±0.031b	0.59±0.12a
Senkyunolide I (%)	3.43	207.1[M+H]$^+$	91 77	38 60	0.044±0.009b	0.022±0.003b	0.025±0.007b	0.105±0.035a	0.061±0.004b	0.039±0.0038b	0.0358±0.0032b
Senkyunolide H (%)	3.51	207.1[M+H]$^+$	91 77	38 60	0.011±0.002b	0.0054±0.0009b	0.0063±0.0006b	0.022±0.007a	0.0061±0.0049b	0.0092±0.0008b	0.0093±0.0006b
Butylphthalide (%)	4.05	191.1[M+H]$^+$	145.1 135	20 25	0.0094±0.0014b	0.014±0.0011ab	0.012±0.001b	0.016±0.0017ab	0.016±0.0013ab	0.015±0.0018ab	0.018±0.01a
Butylidenephalide (%)	4.97	189.1[M+H]$^+$	153.1 115.1	30 35	0.016±0.009b	0.016±0.0014b	0.016±0.0007b	0.021±0.0025a	0.015±0.001b	0.017±0.0007ab	0.017±0.0006ab
Levistolide A (%)	4.95	381.1[M+H]$^+$	191.1	20	0.012±0.009c	0.0212±0.0017b	0.0284±0.0006a	0.0304±0.0025a	0.0308±0.0033a	0.0220±0.0014b	0.0230±0.0008b

The EO yield of *A. sinensis* under PM+CF treatment was significantly higher than control, and produced the maximum essential oil yield that was 49.45% higher over the control. This might be due to the following reasons: First, previous researches showed that the essential oil yield was influenced by crop biomass, the rate of biosynthesis, oil storage glands and number and size of glandular trichomes（Bistgani *et al*., 2018). In this study, the higher essential oil yield ascribed the increased crop biomass under PM+CF treatment. Meanwhile, a significant positive correlation was found between essential oil yield and biomass yield in Pearson's correlation coefficient analysis (R^2=0.913, p<0.05) (Table 3). Further, as mentioned above, the higher nitrogen uptake under PM+CF treatment by the *A. sinensis* plants led to the increase of essential oil yield. Third, phosphorus and potassium participate in various metabolic activities of the plant, which play a vital part in the synthesis of the production of secondary metabolism (Pandey *et al*., 2016).

Different researchers have different views on the effect of combined fertilizers on chemical compositions of essential oil. Some researchers demonstrated that there was no clear trend in the chemical compositions of medicinal plant in different fertilizers treatments (Pandey & Patra, 2015; Singh & Wasnik, 2013). Others showed that integrated fertilizers had active impact on main compound of essential oil (Pandey & Patra, 2015; Emami Bistgani *et al*., 2017a,b). Our results corroborated the earlier finding of Bistgani *et al*., (2018) and Emami Bistgani *et al*., (2017a,b), who reported that integrated fertilizers had active impact on main compound of essential oil. The synthesis of the production of secondary metabolism such as essential oil, depends on the photosynthetic activity (Croteau *et al*., 1972). Combined minerals supply increase photosynthetic and metabolic activities involving in cell division and elongation. Therefore, combined fertilizers increased the content of ligustilide as the major compound of *A. sinensis*.

Conclusion

Our study demonstrated that the use of integrated fertilizers (sheep manure along with chemical fertilizers) in *A. sinensis* produced higher biomass yields, and improved the quality of ferulic acid and EO as well as the soil health. Therefore, this study showed that integrating sheep manure with chemical fertilizers was an ideal nutrient management strategy to enhance the productivity and improve the soil properties of *A. sinensis*.

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