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Analyses of the oil content, fatty acid composition, and antioxidant activity in seeds of *Thlaspi arvense* L. from different provenances and correlations with environmental factors

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Abstract

Background: Pennycress (*Thlaspi arvense* L.) is an annual herbaceous plant of the Cruciferae family that has attracted attention as an oil crop and interseeded cover crop. We collected seeds of pennycress from five provenances in Northeast China, compared their characteristics, i.e. oil content, fatty acid composition, physical, chemical and antioxidant properties, their correlations with environmental factors were also analysed.

Results: There were significant differences in the seed characteristics, oil content, quality indicators and composition among different provenances ($P < 0.05$). The 1000-seed weight ranged from 0.80 to 1.03 g; seed oil content from 28.89 to 42.57%; iodine from 79.19 to 99.09; saponification value from 186.51 to 199.60; peroxide value from 0.07 to 10.60; and acid value from 0.97 to 13.02. The range of seed oil colours were 66.53–78.78 (L*), 4.51–10.29 (a*), and 105.68–121.35 (b*). Erucic acid (C22:1) was the fatty acids with the highest content in pennycress seed oils (31.12–35.31%), followed by linoleic acid (C18:2 16.92–18.95%) and α-linolenic acid (C18:3 14.05–15.34%). The fatty acid 8,11,14-eicosatrienoic acid (C20:3) was detected for the first time in seed oils from Beian city, Panshi city and Kedong county, with contents of 1.13%, 0.84% and 1.03%, respectively. We compare and report for the first time on the radical-scavenging activity of the seed oils of pennycress. The EC50 values of the DPPH radical-scavenging activity and $ABTS^{+}$ radical-scavenging activity of the seed oils from different provenances were 8.65–19.21 mg/mL and 6.82–10.61 mg/mL, respectively. The ferric ion reduction antioxidant capacity (FRAP) ranged from 0.11 to 0.30 mmol Fe^{2+}/g, which is equivalent to 4 mg/mL $FeSO_4$ of pennycress seed oils.

Conclusions: There was a significant correlation between seed characteristics and changes in geographical factors. With increasing longitude, the thickness of seeds, 1000-seed weight, and seed oil content increased, while the acid and peroxide values of the seed oil decreased. As the latitude increased, the 1000-seed weight and seed oil content increased, while the seed oil peroxide value decreased. Furthermore, mean annual temperature and annual rainfall are the two key environmental factors affecting the quality of pennycress.

Keywords: *Thlaspi arvense* L., Seed provenance, Environmental factors, Biodiesel, Seed oil

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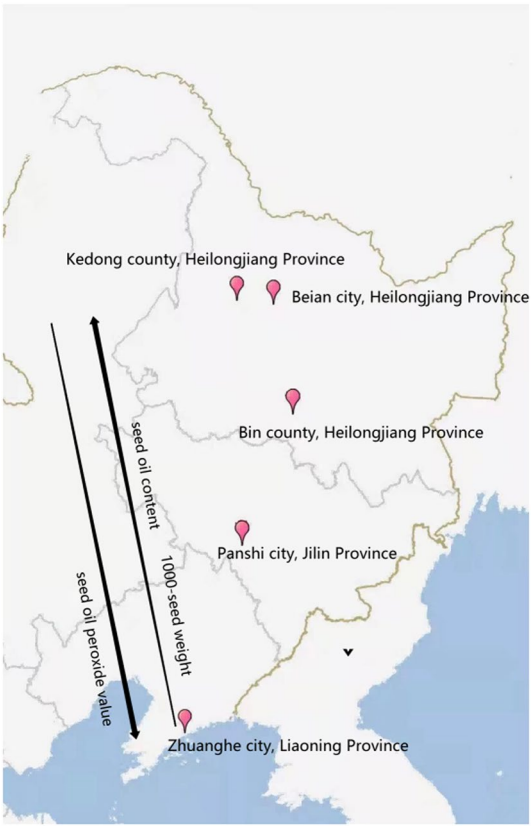
Graphical Abstract



Background

Worldwide, rapid urbanization, industrialization, large-scale transportation and many other man-made activities have led to the depletion of conventional fossil fuels. In addition, the combustion process of fossil fuels is accompanied by a large amount of greenhouse gas emissions, which is one of the main causes of air pollution. Biofuels have great potential for development in coping with global climate change, energy shortages and environmental pollution, and the development and utilization of eco-friendly biofuels (such as biodiesel and bioethanol) have become research hotspots in this field [1, 2]. Biodiesel is a monoalkyl ester of vegetable oil or animal fat that is produced by transesterification with monohydric alcohol (usually methanol). Compared with petroleum diesel, biodiesel has many advantages, such as inherent lubricity, low toxicity, an excellent flash point and biodegradability, a negligible sulfur content, and lower exhaust emissions and is derived from renewable and household raw materials [3, 4]. The purchase of raw materials accounts for 80% or more of the costs related to biodiesel production [5]. The availability of raw materials varies with geography and climate. Currently, the raw materials of biodiesel mainly include rapeseed oil, palm oil, soybean oil and animal fat. Rapeseed oil is mainly used in Europe, palm oil is mainly used in tropical countries, and soybean oil and animal fat are mainly used in the United States. Among rapeseed oils, the field cost of *Camelina sativa* (L.) Crantz is relatively low and has attracted increasing attention [6–9]. China has a vast territory but a large population, and the use of crops as raw materials for biodiesel production is not sustainable based on the environmental conditions in China. The identification of desirable plants to replace food crops is the top priority of China's vigorous development of biodiesel projects.

Pennycress (*Thlaspi arvense* L.) is an annual plant belonging to the Cruciferae family, and has strong environmental adaptability. It is widely distributed worldwide and mainly distributed in Northeast China. In recent years, pennycress has received attention as a potential raw material for biodiesel production [10, 11]. Pennycress seeds have a high oil content (20–36%) [5, 12–14]. Pennycress seed oil is composed of 36% erucic acid [13(Z)-docosadienoic acid] and 20% linoleic acid [9(Z),

12(Z)-octadecadienoic acid] as well as other unsaturated fatty acids [15]. Triacylglycerols are the main reservoir of erucic acid in pennycress seed oil [16]. The high erucic acid component contributes to the excellent low-temperature performance of pennycress as a biodiesel [5]. In addition, erucic acid is an industrial precursor for the synthesis of erucamide, which is very important in the manufacturing of lubricants, surfactants, plasticizers, nylon and surface coatings [17]. The content of erucic acid in pennycress oil can be increased from 34 to 70% through concentration treatment, expanding its sales capacity in the nonfuel chemical market [18]. Through hydrogenation, deoxygenation, isomerization, and hydrocracking reactions, pennycress oil is catalytically converted into renewable fuels [19]. According to the American Society for Testing and Materials (ASTM) D6751 regulations [5], the properties of pennycress seed oil meet the raw material requirements for biodiesel production. Reports have shown that the pennycress seed cake left after oil extraction has potential uses, such as a biological humidifier [20], secondary energy source [19], industrial adhesive [21] and livestock feed [19]. Moreover, the utility of pennycress as an intermediate cover crop and the seed dormancy characteristics have great potential value in an inter-cropping system [22]. For example, pennycress is added in the 2-year corn (first year)-soybean (second year) rotation that is widely used in American agriculture [23]. Studies have shown that without affecting food production, the total annual biofuel production potential of 16.2 million hectares of corn and soybean rotation in the midwestern United States is 15 million cubic metres [19].

During the last decade, significant research has been performed on *Thlaspi arvense* L. and has primarily focused on the following: (a) description and evaluation of the apparent characteristics of pennycress [24, 25]; (b) development and use of different molecular markers to evaluate the genetic variability of pennycress germplasm [26, 27]; (c) domestication of crop rotation of cover crops, multisite testing, and performance evaluation [19, 28–30]; (d) system evaluation and industrial application of pennycress as an oil crop [13, 19, 30, 31]. Exploring and comparing the germplasm resources obtained is the key step in the preservation of germplasm resources, so that high-yield, high-quality, stable and suitable provenances can be selected for practical production [32]. However, little work has been performed to assess the differences in germplasm resources and evaluate the biodiesel performance of *Thlaspi arvense* L. in Northeast China. In this study, pennycress collected from five provenances in Northeast China were selected for comparison. The analysis in current study includes seed morphological traits, seed oil content (especially the fatty acid composition) and antioxidant properties. This investigation explores the relationship between the environmental factors and these properties, and supports the development of pennycress as an oil crop. These findings will support necessary data for improving and selecting provenances of *Thlaspi arvense* L. to produce a greater quantity of high-quality biodiesel.

Materials and methods

Plant materials

In July 2020, seeds were collected from five typical pennycress (*Thlaspi arvense* L.) distribution areas in Northeastern China, and the sampled plant material belonged to the same species. The ecological geographic characteristics of the survey and collection sites are shown in Table 1. Healthy and fresh seeds (2–4 kg) were collected; the seeds were dried in the sun for 1–2 days before further process in the laboratory. The seeds were divided into two parts: one part was stored at 4 °C, and the other part was dried at 40 °C until a constant weight was reached. Then, the seeds were crushed into a fine powder with a medicinal plant crusher and stored at 4 °C.

Determination of seed morphological traits

Thirty seeds were randomly selected from each provenance sample, and the length, width and thickness of each seed were measured with a digital Vernier caliper (Mitutoyo, Japan). The weight of 1000-seed was measured with a digital balance (BSA124S-CW, Beijing

Table 1 Geographical and climatic data of pennycress sampling locations in northern China (AR, annual rainfall; MAT, mean annual temperature)

Provenance	Locality	AR (mm)[a]	MAT (℃)[b]	Altitude (m)
Zhuanghe city, Liaoning Province	39°41′27″ N, 122°56′15″ E	786.5	6.17	33
Bin county, Heilongjiang Province	45°37′07″ N, 127°13′56″ E	956.3	4.42	231.9
Beian city, Heilongjiang Province	47°49′05" N, 127°16′75" E	744.2	3.17	267
Kedong county, Heilongjiang Province	48°01′42″ N, 126°15′12″ E	757.6	3.25	290
Panshi city, Jilin Province	43°16′12″ N, 125°14′09″ E	861	6.17	407

[a,b] AR and MAT were calculated from the data of the climate stations in the county where the sampling point was located

Sartorius Instruments Ltd, China), and each test was repeated three times.

Seed oil extraction

The Soxhlet extraction apparatus was used to extract oil from pennycress seed powder (10 g), the extraction solvent was n-hexane, the liquid-to-material ratio was 1:30, and the extraction time was 8 h. Hexane was removed with a rotary vacuum evaporator (RE-52AA, Shanghai Instruments Ltd, China) in a water bath at 40 °C and then dried in oven (DGG-9240A, Shanghai Senxin Instruments Ltd, China) at 105 °C. Three sub-samples of each collection was analysed, and the average value was taken. Formula (1) was used to calculate the seed oil content, expressed as a dry weight percentage (w/w):

$$\text{Percent oil content} = [W_0/W_1] \times 100. \quad (1)$$

In the formula, W_0 is the weight of the extracted oil, and W_1 is the weight of the sample.

Fatty acid composition analysis

Before analysis of the fatty acid composition, oil samples were subjected to the methyl esterification reaction as follows: 50 mg of crude lipid sample was placed in a centrifuge tube, and 2 mL of 0.5 M sulfuric acid–methanol solution was added and incubated in a 70 °C water bath for 30 min. Then, 1 mL of n-hexane and 6 mL of distilled water were added, and the supernatant was centrifuged. After the supernatant was filtered through a 0.22-μm organic filter, the fatty acid composition was determined by GC–MS [33].

Fatty acid methyl esters were separated and identified by gas chromatography–mass spectrometry (QP2010 Plus, Shimadzu, Japan). The gas chromatography–mass spectrometer was equipped with a split/splitless injector and hydrogen flame ionization detector. The temperature of the injection port was 220 °C, and the carrier gas was helium; the chromatographic column was a 100 m CP-SSil 88 capillary column (Agilent, USA) with an internal diameter of 0.25 mm and a film thickness of 0.20 μm; column flow: 0.47 mL/min, injection volume: 1 μL. The heating program of the column thermostat was as follows: the initial column temperature was 140 °C, which was maintained for 5 min; then, the temperature was increased from 140 °C to 240 °C at a speed of 4 °C/min and maintained at 240 °C for 15 min. The ionization method was EI, the ionization energy was 70 eV, the ion source temperature was 200 °C, the scanning mass range was from 50 to 500 m/z and the sample split ratio was 20:1. The retention time of each peak in the measured sample chromatogram was compared with the retention time of each peak in the chromatogram of 37 fatty acid methyl ester mixed standards (Nu-Chek, USA) to determine the nature of each peak in the sample chromatogram. The area normalization method was used to determine the percentage of each fatty acid relative to the total fatty acid content.

Determination of physical and chemical properties

According to Chinese standards GB/T5530-2005, GB/T5532-2008, GB/T5534-2008, and GB/T2009.227–2016, the acid value (AV), iodine value (IV), saponification value (SV) and peroxide value (PV) of pennycress seed oil were determined. CieLab coordinates (L*, a*, b*) were measured with a colour spectrophotometer (spectrophotometer CM-5, Konica Minolta, Japan). In the three-colour coordinate system, the L* value is a measure of brightness, which ranges from 0 (black) to + 100 (white), the a* ranges from (-) green to (+) red, and the b* value ranges from (-) blue to (+) yellow. As the a* and b* values increase, the colours become more saturated or colourful; however, for neutral colours (white, grey, or black), these values approach zero.

Antioxidant activity

DPPH radical-scavenging activity

The DPPH radical-scavenging activity of the seed oil was measured using the DPPH assay method, with slight modifications [34]. The reaction mixture contained 2 mL of 1 μM DPPH and 2 mL of pennycress seed oil diluted in methanol (ranged from 0.25 to 256 mg/mL). The mixture was shaken vigorously and then allowed to stand in the dark at room temperature for 30 min. The absorbance was measured at 517 nm with an ultraviolet/visible spectrophotometer (Shimadzu UV-2550 series, Japan). Ascorbic acid was used as a positive control. In this experiment, a lower absorbance indicates a stronger scavenging activity. The effective concentration of the extract that can scavenge 50% of DPPH radicals (expressed by EC50; unit: mg/mL) was obtained by plotting the relationship between the scavenging activity and sample concentration. All experiments were performed in triplicate, and the average value was calculated.

$ABTS^+$ radical-scavenging activity

The antioxidant capacity of the measurement sample reduces the blue 2,2′-azido-3-ethylbenzothiazolin-6-sulfonic acid ($ABTS^+$) radical to its uncoloured form, and the measurement method was slightly modified [35]. $ABTS^+$ reagent was generated by reacting 7 mmol/L ABTS solution and 2.45 mmol/L potassium persulfate solution at room temperature in the dark for 24 h. Then, methanol solution was used to dilute the $ABTS^+$ reagent to an absorbance of 0.70 ± 0.02 at 734 nm. The resulting $ABTS^+$ solution (2 mL) was added to samples (1 mL) of different concentrations (0.25–128 mg/mL), and the

absorbance was measured at 734 nm. Ascorbic acid was used as a positive control. The effective concentration (EC50) was determined by plotting the percentage reduction in absorbance according to the concentration, which was defined as the concentration of the extract (mg/mL) at which $ABTS^+$ radicals were reduced by 50%. All experiments were performed in triplicate, and the average value was calculated.

Determination of the iron reduction/antioxidation ability (FRAP)

FRAP analysis was carried out according to [36, 37] previous studies with some modifications. The stock solution included 0.3 mol/L acetate buffer ($C_2H_3NaO_2$ and $C_2H_4O_2$; pH = 3.6), 10 mM TPTZ solution (40 mM HCl) and 20 mM $FeCl_3$ solution. The fresh working solution was prepared by mixing 250 mL of acetate buffer, 25 mL of TPTZ solution, and 25 mL of $FeCl_3$ solution. Then, 2 mL of 4 mg/mL pennycress seed oil was mixed with 3 mL of the FRAP solution and allowed to react in the dark at 37 °C for 10 min, after which the absorbance was measured at 593 nm. Different concentrations of ferrous sulphate standard solutions (2 mL of 0.1, 0.2, 0.3, 0.4 and 0.5 mmol Fe^{2+}/L) were mixed with 3 mL of FRAP solution and allowed to react in the dark at 37 °C for 10 min, after which the absorbance was measured at 593 nm, which is used to make a standard curve. The antioxidant activity of the seed oil is expressed in mmol $FeSO_4$ E.

Statistical analysis

All data are expressed as the mean ± standard deviation (SD) of three experiments, and one-way analysis of variance (ANOVA) was performed. The IBM SPSS Statistics 25 software package was used to perform Duncan's multiple range test, compare significant differences ($P < 0.05$) and conduct Pearson correlation analyses.

Results and discussion

Seed morphology and 1000-seed weight

As shown in Table 2, the average seed length ranged from 1.73 (Panshi city) to 1.89 mm (Kedong county), the average seed width ranged from 1.17 (Panshi city) to 1.35 mm (Bin county), the average seed thickness ranged from 0.62 (Zhuanghe city) to 0.73 mm (Bin county), and the average seed weight per thousand seeds ranged from 0.80 (Zhuanghe city) to 1.03 g (Beian county). Seeds with a larger mass of one thousand grains have a higher nutrient content for seed germination [38]. Kayla Altendorf studied 41 provenances of pennycress at three locations in Minnesota, and the average 1000-seed weight was 0.97–1.12 g [28]. The 1000-seed weight of pennycress seeds studied by V. S. Dolya was 0.79 g [25], and the average 1000-seed weight of pennycress seeds in this study fell within these ranges.

Variations in the phenotypic traits of pennycress seeds are mainly affected by latitude and longitude and annual average temperature. As shown in Table 8, the 1000-seed weight was significantly negatively correlated with the annual average temperature; as longitude increased, seed thickness increased and the 1000-seed weight increased. With increasing latitude, the 1000-seed weight increased, which means that the 1000-seed weight of pennycress in Beian city ranked the highest. Qiwentao studied the relationship between the seed traits of *Bupleurum chinense* DC. from different provenances and the environment, and showed that with increasing longitude, the width and thickness of seeds, as well as the 1000-seed weight, increased [39].

Oil content

The oil content of seeds is an important indicator to measure the quality of raw materials used for biodiesel. The geographical location of a provenance has been shown to have a significant impact on the oil content of pennycress [40]. As shown in Table 2, the average seed oil content of pennycress ranged from 28.89 (Zhuanghe city) to 42.57% (Beian city), and the largest difference between provenances was 13.69%. Kayla Altendorf used pulsed nuclear magnetic resonance (pNMR) technology to measure the average oil content of 41 seeds of pennycress from different provenances; the range was 30.0–33.3%, and the difference between provenances was 3.3% [28]. According to John C. Sedbrook's research, the oil content of 80 wild materials from Western Illinois University was

Table 2 Morphological traits and oil content of *Thlaspi arvense* L. seeds from five provenances

Provenance	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	1000-seed weight (g)	Oil content (%)
Zhuanghe city	1.86 ± 0.01^{a}	1.28 ± 0.01^{c}	0.62 ± 0.01^{c}	0.80 ± 0.01^{d}	28.89 ± 1.76^{d}
Bin county	1.86 ± 0.04^{a}	1.35 ± 0.02^{a}	0.73 ± 0.02^{a}	1.03 ± 0.00^{a}	39.53 ± 0.81^{b}
Beian city	1.89 ± 0.01^{a}	1.29 ± 0.00^{bc}	0.71 ± 0.01^{a}	1.03 ± 0.00^{a}	42.57 ± 0.69^{a}
Kedong county	1.89 ± 0.02^{a}	1.32 ± 0.03^{ab}	0.70 ± 0.02^{a}	0.99 ± 0.00^{b}	38.58 ± 0.44^{bc}
Panshi city	1.73 ± 0.01^{b}	1.17 ± 0.00^{d}	0.65 ± 0.01^{b}	0.86 ± 0.00^{c}	36.71 ± 0.47^{c}

The data are expressed as the mean ± SD. Different lowercase letters indicate significant differences at the 5% probability level

13.5–38.7%, and the difference between provenances was 25.2%; the U.S. Department of Agriculture's 34 wild species of pennycress had an oil content of 24.7–38.7%, and the difference between provenances was 14% [41]. The oil content of pennycress from different provenances in this study was higher than that of oil crops; for example, the oil content of *Helianthus annuus* L. seeds of 86 different provenances in Seiler's study ranged from 21.1to 27.5% [42], and the oil content of *Glycine max* (L.) Merr. in Xiyu Li's study was 20% [43]. Based on the oil content figures above, pennycress seed oil has good biodiesel production potential.

Longitude and latitude were significantly positively correlated with the seed oil content, and the correlation coefficients were 0.98 ($P<0.01$) and 0.90 ($P<0.05$), respectively. With increasing longitude, the seed oil content increased gradually, and the highest oil content was found in the pennycress seeds in Beian city, which may be partially due to the large temperature difference between day and night. It is possible to cultivate and domesticate pennycress seeds in Beian city through targeted induction of local genome mutations and targeted genome editing to increase the seed size and oil content for production of biodiesel. For example, overexpression of WRINKLED1 (WRI1), HAIKU2 (IKU2) and KLUH (KLU) in *Arabidopsis thaliana* (L.) Heynh. increased the seed oil content and seed size, while loss-of-function mutations GLABRA2 (GL2) and MUCILAGE-MODIFIED 4 (MUM4) demonstrated increased seed oil content [41].

Fatty acid composition analysis

The fatty acid composition affects the industrial applications of oils, such as lubricants and biodiesel [44]. Table 3 shows that the composition and content of fatty acids in the seed oil of pennycress from five provenances were significantly different ($P<0.05$). Panshi city and Kedong county seed oils contained 14 fatty acids, and Beian city, Zhuanghe city and Bin county seed oils contained 13 fatty acids. 8,11,14-eicosatrienoic acid (C20:3) was detected for the first time in the seed oil from Beian city, Panshi city and Kedong county, and the contents were 1.13%, 0.84% and 1.03%, respectively.

Pennycress seed oil fatty acids were mainly composed of erucic acid (C22:1), linoleic acid (C18:2), and linolenic acid (C18:3), which is consistent with previous studies [5, 28]. The provenance with the highest erucic acid content in seed oil was in Bin county (35.31%), followed by Zhuanghe city (34.48%), Beian city (33.51%), Kedong county (32.77%), and Panshi city (31.12%). The high erucic acid component contributes to the excellent low-temperature performance of pennycress as a biodiesel [5]. The erucic acid content in rapeseed oil fatty acids produced by *Brassica* L. rape (*Brassica napus* L., *Brassica rapa* L., *Brassica juncea* (Linnaeus) Czernajew) was 45% [37]. C22:1 has important value in industrial applications; for these purposes, it is best to increase the content of erucic acid and its derivatives in pennycress seed oil to the highest possible level to improve the economic value. However, *Thlaspi arvense* L. is an undesirable oilseed crop for human consumption because its seeds contain glucosinolates and particularly high levels of erucic acid, which are toxins of biological origin [45]. The linoleic acid contents in the seed oil from Beian city, Zhuanghe city, Panshi city, Bin county and Kedong county were 18.52%, 18.95%, 18.88%, 16.92%, 18.52%, and the linolenic acid contents were 15.34%, 14.05%, 14.99%, 14.96% and 14.19%, respectively. The values of other fatty acids are as follows: oleic acid, 9.45–12.81%; eicosenoic acid,

Table 3 Fatty acid content (wt. %) in the oil of *Thlaspi arvense* L. seeds from five provenances

Provenance	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
Zhuanghe city	0.36 ± 0.00^{a}	4.96 ± 0.03^{a}	0.51 ± 0.00^{a}	0.76 ± 0.01^{a}	10.51 ± 0.12^{d}	18.95 ± 0.09^{a}	14.05 ± 0.05^{d}
Bin county	0.14 ± 0.00^{d}	3.53 ± 0.02^{d}	0.43 ± 0.00^{d}	0.56 ± 0.01^{d}	11.23 ± 0.01^{b}	16.92 ± 0.07^{c}	14.96 ± 0.03^{b}
Beian city	0.20 ± 0.00^{c}	3.69 ± 0.05^{c}	0.49 ± 0.01^{b}	0.59 ± 0.02^{c}	9.45 ± 0.10^{e}	18.52 ± 0.09^{b}	15.34 ± 0.05^{a}
Kedong county	0.21 ± 0.00^{b}	4.52 ± 0.01^{b}	0.45 ± 0.01^{c}	0.78 ± 0.01^{a}	10.76 ± 0.08^{c}	18.52 ± 0.05^{b}	14.19 ± 0.09^{c}
Panshi city	0.20 ± 0.01^{c}	3.71 ± 0.03^{c}	0.42 ± 0.00^{e}	0.63 ± 0.01^{b}	12.81 ± 0.43^{a}	18.88 ± 0.13^{a}	14.99 ± 0.04^{b}
Provenance	**C20:0**	**C20:1**	**C20:2**	**C20:3**	**C22:1**	**C22:3**	**C24:1**
Zhuanghe city	0.23 ± 0.01^{a}	9.24 ± 0.10^{e}	2.75 ± 0.08^{c}	nd	34.48 ± 0.31^{b}	0.28 ± 0.00^{b}	2.93 ± 0.01^{b}
Bin county	0.23 ± 0.01^{a}	9.69 ± 0.01^{d}	3.00 ± 0.05^{b}	nd	35.31 ± 0.01^{a}	0.30 ± 0.02^{a}	3.72 ± 0.02^{a}
Beian city	0.18 ± 0.01^{b}	10.65 ± 0.06^{b}	3.29 ± 0.12^{a}	1.13 ± 0.08^{a}	33.51 ± 0.28^{c}	nd	2.70 ± 0.07^{c}
Kedong county	0.22 ± 0.01^{a}	10.35 ± 0.05^{c}	3.17 ± 0.05^{a}	1.03 ± 0.06^{a}	32.77 ± 0.20^{d}	0.30 ± 0.01^{a}	2.73 ± 0.05^{c}
Panshi city	0.19 ± 0.00^{b}	11.46 ± 0.07^{a}	2.31 ± 0.03^{d}	0.84 ± 0.05^{b}	31.12 ± 0.09^{e}	0.24 ± 0.00^{c}	2.21 ± 0.03^{d}

The data are expressed as the mean ± SD. Different lowercase letters indicate significant differences at the 5% probability level. nd = not detected

9.24–11.46%; and palmitic acid, 3.53–4.96%, and the content of nervonic acid (C24:1) was 2.21–3.72%.

There are three main fatty acids in triglycerides: saturated fatty acids (Cn:0), monounsaturated fatty acids (Cn:1) and polyunsaturated fatty acids with two or three double bonds (Cn:2,3) [32]. Table 4 shows the percentages of various compounds in pennycress seed oil. The seed oil was mainly composed of total unsaturated fatty acids (UFA_S), and the content was 93.70–95.55%. Compared with total unsaturated fatty acids, total saturated fatty acids (SFA_S) had a very low content of 4.45–6.30%. The provenance with the highest total unsaturated fatty acid content in seed oil was Bin county, followed by Panshi city and Beian city. The provenance with the highest total saturated fatty acid content in seed oil was Zhuanghe city, i.e. 6.30%. The ratio of polyunsaturated fatty acids/monounsaturated fatty acids (PUFAs/MUFAs) is an important parameter of oil oxidation stability in highly unsaturated oils [32]. Bin county seed oil had the lowest ratio of polyunsaturated to monounsaturated fatty acids, at 0.58, which may be due to its high oleic acid content, followed by Zhuanghe city (0.63), Panshi city (0.64) and Kedong county (0.65); the highest ratio was measured in Beian city (0.67). The oleic acid/linoleic acid (O/L) ratio is considered to be an important criterion for evaluating the quality of seed oil [46]. The highest O/L ratio occurred in Panshi city (0.68), followed by Bin county (0.66), Kedong county (0.58), Zhuanghe city (0.55), and Beian city (0.51). The PUFA/MUFA and O/L ratios indicated that Bin county and Panshi city seed oils had high oxidation stability.

Physicochemical properties of seed oil

The physicochemical properties of pennycress seed oil from different provenances are shown in Table 5. The a* value of the oil sample was low (4.51–10.29), and the b* value was high (105.68–121.35), indicating that the colour of the seed oil was yellow-green. There were differences in the colour of seed oil from different provenances (Additional file 1: Figure S1). The colour of vegetable oils depends on the pigments. Pigments in oils play an important role in the oxidation stability of the oil in addition to their colouring properties [47]. The iodine value indicates the degree of unsaturation of the fat; the higher the iodine value, the higher the degree of unsaturation of the fat. Based on the iodine value, grease can be divided into three types: dry oil (the iodine value is higher than 130), semidry oil (the iodine value is between

Table 4 Various nutritional parameters based on the fatty acid profile of *Thlaspi arvense* L. seed oil from five provenances

Provenance	∑SFAs	∑UFAs	∑MUFAs	∑PUFAs	PUFAs/MUFAs (ratio)	C18:1/C18:2 (ratio)
Zhuanghe city	6.30 ± 0.04^{a}	93.70 ± 0.04^{e}	57.67 ± 0.11^{c}	36.03 ± 0.08^{c}	0.63	0.55
Bin county	4.45 ± 0.03^{e}	95.55 ± 0.03^{a}	60.37 ± 0.04^{a}	35.18 ± 0.06^{d}	0.58	0.66
Beian city	4.65 ± 0.05^{d}	95.07 ± 0.05^{c}	56.79 ± 0.26^{d}	38.28 ± 0.22^{a}	0.67	0.51
Kedong county	5.73 ± 0.02^{b}	94.27 ± 0.02^{d}	57.05 ± 0.12^{d}	37.22 ± 0.12^{b}	0.65	0.58
Panshi city	4.72 ± 0.03^{c}	95.27 ± 0.03^{b}	58.02 ± 0.16^{b}	37.26 ± 0.18^{b}	0.64	0.68

∑SFAs indicates the total concentration of saturated fatty acids; ∑UFAs indicates the total concentration of unsaturated fatty acids; ∑MUFAs indicates the total concentration of monounsaturated fatty acids; ∑PUFAs indicates the total concentration of polyunsaturated fatty acids. C18:1/C18:2 indicates the ratio of oleic acid to linoleic acid. The value is expressed as the average (±) standard error of three determinations. Different lowercase letters indicate significant differences at the 5% probability level

Table 5 Physicochemical properties of *Thlaspi arvense* L. seed oil from five provenances

Physicochemical properties	Zhuanghe city	Bin county	Beian city	Kedong county	Panshi city
Iodine value (g iodine/100 g fat)	79.19 ± 7.03^{c}	99.09 ± 0.80^{a}	90.84 ± 2.02^{ab}	83.23 ± 4.09^{bc}	97.28 ± 4.41^{a}
Saponification value (mg KOH/g)	193.52 ± 0.81^{ab}	199.60 ± 2.14^{a}	189.32 ± 3.24^{b}	187.68 ± 4.67^{b}	186.51 ± 5.67^{b}
Peroxide value (mEq/kg)	10.60 ± 1.69^{a}	0.13 ± 0.03^{cd}	0.07 ± 0.05^{d}	1.63 ± 0.08^{c}	4.03 ± 0.13^{b}
Acid value (mg KOH/g)	13.02 ± 1.52^{a}	3.33 ± 0.34^{b}	0.97 ± 0.07^{c}	2.24 ± 0.22^{bc}	2.21 ± 0.07^{bc}
Colour					
L*	78.09 ± 4.32^{ab}	66.53 ± 3.75^{c}	78.78 ± 1.48^{a}	67.76 ± 4.08^{c}	71.79 ± 0.80^{bc}
a*	7.61 ± 0.27^{c}	8.47 ± 0.90^{b}	4.51 ± 0.06^{e}	10.29 ± 0.11^{a}	6.07 ± 0.07^{d}
b*	121.35 ± 5.97^{a}	105.68 ± 4.81^{c}	119.25 ± 1.78^{ab}	111.10 ± 6.04^{bc}	111.84 ± 0.91^{bc}

L*, a*, b* are the chroma values representing the colour of the oil. The L* value is a measure of brightness, which ranges from 0 (black) to + 100 (white), the a* ranges from (−) green to (+) red, and the b* value ranges from (−) blue to (+) yellow. The value is expressed as the average (±) standard error of three determinations. Different lowercase letters indicate significant differences at the 5% probability level

100 and 130), and nondrying oil (the iodine value is less than 100). The iodine values of pennycress seed oil from five provenances were all lower than 100, making them all nondrying oils, ranging from 79.19 (Zhuanghe city) to 99.09 (Bin county). A relatively high iodine value of oil means that the oil contains relatively high amounts of unsaturated fatty acids [48], which is consistent with the results of the study shown in Table 4. The saponification value is important for testing the quality of fats, and a low saponification value occurs because fat contains a certain amount of impurities that cannot be saponified. The higher the saponification value, the lower the average molecular weight of the oil. The saponification value of pennycress seed oil ranged from 186.51 (Panshi city) to 199.60 (Bin county). The peroxide value is an indicator of the degree of oxidation of oils and fatty acids, which can reflect the degree of rancidity of oils. Some small molecules produced by rancid oils have adverse effects on the human body; generally speaking, the higher the peroxide value, the more serious the rancidity [37]. Table 5 shows that Beian city seed oil had the lowest peroxide value, 0.07 mEq/kg, followed by Bin county (0.13 mEq/kg), Kedong county (1.63 mEq/kg), and Panshi city (4.03 mEq/kg). Zhuanghe city seed oil had the highest peroxide value of 10.60 mEq/kg. The FDA (1974) stipulated a maximum level of 10 mEq/kg petroleum at room temperature [49]. Except for Zhuanghe city seed oil, the oils from the four other provenances are high-quality oils. The content of free fatty acids in oils is usually expressed by acid value, which is one of the main parameters to measure the quality of oils. Generally, oils with acid values higher than 6 are not suitable for consumption. The acid value of the seed oil was the lowest in Beian city (0.97), followed by Panshi city (2.21), Kedong county (2.24), Bin County (3.33), and Zhuanghe city (13.02). Longitude and latitude were significantly negatively correlated with the seed oil peroxide value, and the correlation coefficients were − 0.99 ($P<0.01$) and − 0.90 ($P<0.05$), respectively. There was a significant negative correlation between longitude and the seed oil acid value ($r=-0.91$, $P<0.05$). With increasing longitude, the seed oil from Beian city had a low peroxide value and low acid value, indicating high-quality oil.

Antioxidant activity

DPPH free radical-scavenging test

DPPH is a stable free radical that is widely used to study the free radical-scavenging activity of natural antioxidants [50]. In the presence of a free radical scavenger, a single electron of DPPH is captured, and its colour becomes lighter. The absorbance value at 517 nm decreased linearly. The decrease in the absorbance level indicates an increase in antioxidant capacity to evaluate

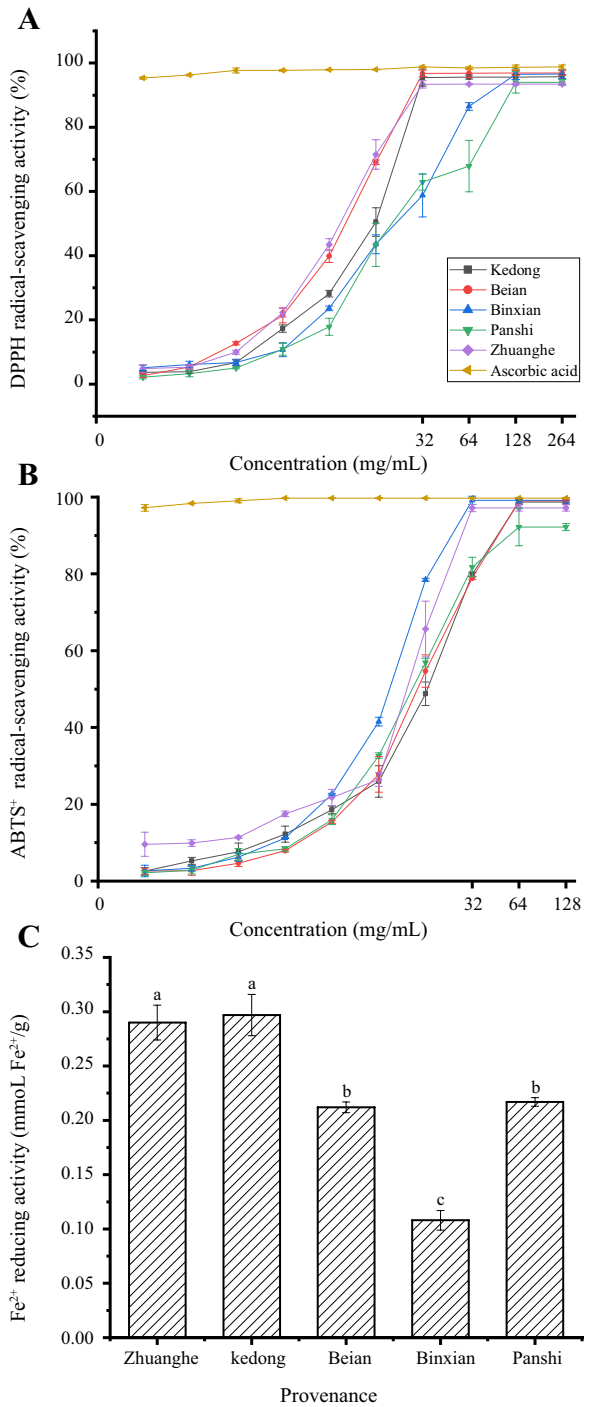


Fig. 1 Comparison of **A** DPPH radical-scavenging activity, **B** $ABTS^{+}$ radical-scavenging activity and **C** Fe^{2+} reducing activity. The values are expressed as the mean ± standard deviation. Bars not sharing a letter are significantly different at $P<0.05$ based on Duncan's test. a–c Means are significantly different ($P<0.05$) based on Duncan's test

the antioxidant capacity of the test samples. Figure 1A shows the DPPH free radical-scavenging activity of pennycress seed oil from different provenances. As the concentration of seed oil increased, the scavenging rate gradually increased and stabilized. The concentration of seed oil from Beian city, Kedong county and Zhuanghe city reached the maximum clearance rate at 32 mg/mL, and Beian city > Kedong county > Zhuanghe city. The concentration of seed oil from Bin county and Panshi city reached the maximum clearance rate at 128 mg/mL, and Bin county > Panshi city. Table 6 shows that the EC50 values of DPPH radicals for Kedong county, Beian city, Bin county, Panshi city and Zhuanghe city were 10.64 mg/mL, 8.65 mg/mL, 15.17 mg/mL, 19.21 mg/mL and 8.70 mg/mL, respectively. The lower the EC50 value was, the higher the DPPH free radical-scavenging activity. The results showed that seed oil from Beian city and Zhuanghe city had the highest DPPH free radical-scavenging activity, followed by Kedong county, Bin county and Panshi city. The oxidative stability of vegetable oils depends in part on the fatty acid composition. It was reported that oleic acid oxidizes at a rate 50 times slower than linoleic acid. Thus, the DPPH free radical-scavenging activity of seed oil in Beian city is the largest, partly because the oils have the highest level of oleic acid [51]. A study conducted by Hung-Chih Ting showed that the EC50 value of *Hippophae rhamnoides* L. seed oil for scavenging DPPH free radicals was 7.37 mg/mL [34]. Chao-Chin Hu's research showed that the EC50 values of α-tocopherol, all-trans zeaxanthin, all-trans lutein, all-trans β-carotene, and all-trans carotene for scavenging DPPH free radicals were 2.88, 22.82, 23.04, 24.17, and 24.54 mg/mL, respectively [52]. The results showed that the DPPH free radical-scavenging activity of pennycress seed oil was lower than that of *Hippophae rhamnoides* L. seed oil and α-tocopherol but higher than that of all-trans zeaxanthin, all-trans lutein, all-trans β-carotene, and all-trans carotene. Tao Bao's research showed that the antioxidant capacity of *Morus alba* L. (measured by DPPH) was positively correlated with the total phenol content [53]. However, other research showed a positive correlation between the radical-scavenging activity of the selected oils and their levels of unsaponifiables and phytosterols, while a negative relationship was noted with the amounts of phenolics and tocopherols [54]. The different DPPH free radical activities of pennycress seed oil from different provenances may be due to differences in the total phenol content in seed oil, and the causes of the differences should be studied further.

ABTS test

ABTS can be oxidized by $K_2S_2O_8$ to generate the blue-green free radical cation $ABTS^{\bullet+}$. $ABTS^{\bullet+}$ is quite stable, with a maximum absorption peak at 734 nm. In the presence of antioxidants, $ABTS^{\bullet+}$ reacts with it and becomes colourless ABTS. Figure 1B shows that the seed oil concentration in Bin county and Zhuanghe city reached the maximum removal rate at 0.25–32 mg/mL and that Bin County > Zhuanghe city. The seed oil concentration in Kedong county, Beian city and Panshi city reached the maximum clearance rate at 0.25–64 mg/mL. Table 6 shows that the minimum EC50 values of ABTS free radicals were measured in seed oil from Bin county (6.82 mg/mL) and Zhuanghe city (7.41 mg/mL). There was no difference in the EC50 of ABTS free radicals among Kedong county, Beian city and Panshi city seed oils ($P < 0.05$). The results showed that the ABTS radical-scavenging activities of Bin county and Zhuanghe city seed oils were the highest. Guorong Du's study showed that the ABTS free radical-scavenging activity of *Actinidia chinensis* Planch. Was positively correlated with the content of total polyphenols and vitamin C [36]. Tao Bao showed that mulberry ABTS free radical-scavenging activity was positively correlated with the total phenol content, total flavonoid content, proanthocyanidins, cyano-3-O-glucoside, anthocyanin-3-O-rutin, and rutin [53]. Moreover, the phenolic compound content and profile in plant oil generally depends on the variety, environmental conditions, extraction methods, and storage conditions [47]. The factors that influence the ABTS free radical-scavenging activity of pennycress seed oil from different provenances will be determined later.

FRAP test

FRAP is a test for the determination of reducing power, which can evaluate the ability of natural antioxidants to provide electrons or hydrogen atoms through the process of converting iron ions into ferrous forms [34]. Table 6

Table 6 EC50 of DPPH and ABTS and the $FeSO_4$ equivalent of FRAP in *Thlaspi arvense* L. seed oil from five provenances

	Zhuanghe seed oil (mg/mL)	Binxian seed oil (mg/mL)	Beian seed oil (mg/mL)	Kedong seed oil (mg/mL)	Panshi seed oil (mg/mL)
ABTS (EC_{50})	7.41 ± 0.56^{b}	6.82 ± 0.12^{b}	10.27 ± 0.51^{a}	10.13 ± 0.20^{a}	10.61 ± 0.66^{a}
DPPH(EC_{50})	8.70 ± 0.21^{d}	15.17 ± 0.37^{b}	8.65 ± 0.25^{d}	10.64 ± 0.42^{c}	19.21 ± 1.68^{a}
FRAP($mmolFe^{2+}/g$)	0.29 ± 0.02^{a}	0.11 ± 0.01^{c}	0.21 ± 0.01^{b}	0.30 ± 0.02^{a}	0.22 ± 0.00^{b}

The value is expressed as the average (±) standard error of three determinations. Different lowercase letters indicate significant differences at the 5% probability level

shows that the $FeSO_4$ equivalents of the seed oils in Kedong county, Zhuanghe city, Panshi city, Beian city and Bin county were 0.30, 0.29, 0.22, 0.21 and 0.11, respectively. Bin county seed oil had the lowest iron reduction ability. The Fe^{2+} reduction ability of the *Sargassum* crude lipid extract was 0.69 mmol Fe^{2+}/g [55], which is higher than that of pennycress seed oil. However, among the 56 Chinese medicinal materials studied by Feng-Lin Song, the iron reduction abilities of *Angelica dahurica* (Fisch. ex Hoffm.) Benth., *Arisaema erubescens* (Wall.) Schott, *Aster tataricus* L.f., *Sinapis alba* Linnaeus, and *Bupleurum chinense* DC. were lower than that of pennycress [56]. This result indicates that pennycress seed oil has a good iron ion reduction ability.

Correlation between seed phenotypic traits and oil content

Table 7 shows the correlation between the seed length, width, thickness, 1000-seed weight and oil content. There was a significant positive correlation between the seed thickness and the 1000-seed weight ($r=0.99$, $P<0.01$), the seed length and seed width ($r=0.90$, $P<0.05$), and the 1000-seed weight and seed oil content ($r=0.90$, $P<0.05$). Kumar R. obtained a significant positive correlation between the 100-seed weight and oil content in a study that examined the difference in seed traits and oil content of *Jatropha curcas* L., and seed length was positively correlated with the 100-seed weight, oil content, seed thickness, and seed width [57]. GR Rao studied *Jatropha curcas* L. and found a significant correlation between the seed weight, oil content and seed width [58]. Traits that are likely to show significant correlations are controlled by tightly linked genes.

Correlation of seed phenotypic traits and seed oil characteristics with geographic and climatic factors

The correlations between longitude, latitude, annual rainfall (AR), mean annual temperature (MAT), altitude and seed phenotypic traits, oil content, fatty acid composition, physical, chemical, and antioxidant properties were analysed (Table 8). Although altitude had no correlation with these indicators, the mean annual temperature was significantly negatively correlated with the 1000-seed weight ($r=-0.90$, $P<0.05$), and annual rainfall was significantly positively correlated with total

Table 7 Correlation analysis between seed phenotypic traits and oil content in five provenances

Traits	Seed length	Seed width	Seed thickness	1000-seed weight	Oil content
Seed length	1				
Seed width	0.90*	1			
Seed thickness	0.48	0.63	1		
1000-seed weight	0.55	0.63	0.99**	1	
Oil content	0.22	0.24	0.88*	0.90*	1

*Significant at the 5% probability level

**Significant at the 1% probability level

Table 8 Correlation of seed characteristics and seed oil characteristics with geographical and climatic factors

	Seed length	Seed width	Seed thickness	1000-seed weight	Oil content	Iodine value	Saponification value	Peroxide value
AR	− 0.38	0.04	0.24	0.09	0.05	0.74	0.66	− 0.19
MAT	− 0.73	− 0.64	− 0.83	− 0.90*	− 0.78	0.02	0.07	0.77
Altitude	− 0.51	− 0.45	0.37	0.34	0.66	0.65	− 0.54	− 0.64
Longitude	0.24	0.35	0.95*	0.94*	0.98**	0.64	0.05	− 0.99**
Latitude	0.41	0.39	0.85	0.89*	0.90*	0.25	− 0.24	− 0.90*
	Acid value	**∑SFAs**	**∑UFAs**	**∑MUFAs**	**∑PUFAs**	**ABTS**	**DPPH**	**FRAP**
AR	− 0.08	− 0.53	0.63	0.97**	− 0.74	− 0.55	0.72	− 0.82
MAT	0.62	0.25	− 0.17	0.25	− 0.40	− 0.28	0.44	0.07
Altitude	− 0.88	− 0.66	0.68	− 0.04	0.47	0.73	0.68	− 0.26
Longitude	− 0.91*	− 0.80	0.77	0.20	0.25	0.27	0.19	− 0.60
Latitude	− 0.86	− 0.46	0.41	− 0.17	0.46	0.49	− 0.06	− 0.17

*Significant at the 5% probability level

**Significant at the 1% probability level

saturated fatty acids ($r=0.97$, $P<0.01$). Longitude was significantly positively correlated with the seed thickness, 1000-seed weight, and oil content (the correlation coefficients were 0.95 ($P<0.05$), 0.94 ($P<0.05$), and 0.98 ($P<0.01$), respectively), but was significantly negatively correlated with the peroxidation value and acid value of seed oil, and the correlation coefficients were − 0.99 ($P<0.01$) and − 0.91 ($P<0.05$), respectively. Latitude was significantly positively correlated with the 1000-seed weight and oil content, and the correlation coefficients were 0.89 ($P<0.05$) and 0.90 ($P<0.05$), respectively, but was significantly negatively correlated with the seed oil peroxide value ($r=-0.90$, $P<0.05$). Pennycress seed and oil characteristics showed obvious geographical changes; as the longitude increased, the seed thickness increased, the 1000-seed weight increased, the seed oil content increased, and the seed oil acid value and peroxide value decreased. As the latitude increased, the 1000-seed weight increased, the oil content of seeds increased, and the peroxide value of seed oil decreased. Seiler's research showed that the oil content of 13 species of 215 wild *Helianthus annuus* L. seeds in the United States was significantly positively correlated with longitude and latitude, with correlation coefficients of 0.4 and 0.28, respectively [42]. However, Yunxia Ma studied 26 provenances of *Xanthoceras sorbifolium* Bunge in northern China and found no correlation between longitude and latitude and the seed oil content [46]. The correlation between the seed oil content and latitude and longitude may be related to the diversity of wild plants in the study area. Studies have shown that understanding the impact of environmental factors on the characteristics of pennycress seeds and seed oil is helpful for the selection of high-quality provenances and the development and application of biofuels.

Conclusion

In this study, the seed quality of pennycress from five provenances in Northeast China was evaluated by comparing the phenotypic traits. The seed oil content, physicochemical properties, antioxidant activity and fatty acid composition were analysed. Our results showed that seeds collected from Beian city had a higher 1000-seed weight and seed oil content, with lower peroxide and acid values in seed oil, than seeds from other provenances. The seed oils from Panshi city and Bin county had lower PUFA/MUFA values and higher O/L ratios. Correlation analysis showed that longitude and latitude had significantly positive impacts on the seed thickness, 1000-seed weight, and seed oil content. However, they showed significantly negative impacts on the peroxide value and acid value of the seed oil. The mean annual temperature had a significantly negative impact on the 1000-seed weight; annual rainfall positively impacted the monounsaturated fatty acid content of the seed oil. In conclusion, the seed quality of pennycress varies due to geographical environment.

Abbreviations

AR: Annual rainfall; MAT: Mean annual temperature; GC: Gas chromatography; MS: Mass spectrometry; ∑SFAs: The total concentration of saturated fatty acids; ∑UFAs: The total concentration of unsaturated fatty acids; ∑MUFAs: The total concentration of monounsaturated fatty acids; ∑PUFAs: The total concentration of polyunsaturated fatty acids.

Supplementary Information

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Additional file 1: Figure S1. The seed oil colour of *Thlaspi arvense* L. from five provenances (1) is Beian city, (2) is Bin County, (3) is Panshi city, (4) is Kedong County, and (5) is Zhuanghe city.

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Authors' contributions

LJG designed, conducted experiments, processed data and wrote manuscripts. CM conducted experiments. ZYH contributed to the design and writing of the manuscript. ZBJ contributed to the writing of this manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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