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A GC-MS Characteristic Analysis of Hybrirock and Pactol Seeds Harvested in Erbil/Iraq

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ABSTRACT

The research project designed for the first time on comparison of Hybrirock seeds and pactol seeds of Brssica napus L. A plant affiliation of Brassicaceae family. The objective of this study was to phytochemically analyzing of France purchased Hybrirock and local pactol seeds by Gas chromatography mass spectrophotometry and comparison of their essential oil components in relation with previous works. The French purchased hybrirock seeds and local pactol seeds were planted in Gerda rasha field Grdarash field, College of Agriculture, Salahaddin University-Erbil and seeda were stored for later chemical analysis. The GC-MS detections showed that the most abundant phytochemical constituents were E, E, 2, 4-Decadienal (22.3% for Hybrirock seed oils and 15.64% for pactol seed) and Hexanal (18.37 for hybrirock seed and 8.72% for Pactol seed oils), followed by 2-Undecenal (7.66% for hybrirock seed and 14.27% for pactol seed oils). Our study demonstrated that both studied genotypes of same species contained different important chemicals and beneficial polyunsaturated fatty acids however glucosinolates were significantly higher in pactol seeds in compare with hybrirock seeds, making pactol seeds less edible and more suitable as nematicides or as precursor of Bio-fuels and the two tested genotype seeds have shown sufficient hydrocarbon content which can be used as a substitute of petrol hydrocarbon. While hybrirock seeds with higher fatty acid contents and lower glucosinolates possessing more edibility features to be used as food products and medical agents.

KEYWORDS: Brassica napus L.; Essential oils; GC-MS; Hybrirock and pactol seeds; Glucosinolates.

الخلاصة

تم تصميم المشروع البحثي لأول مرة على مقارنة بذور Hybrirock وبذور باكتول من Brssica napus L. كان الهدف من هذه الدراسة هو التحليل الكيميائي للنباتات، تم الحصول على Hybrirock من فرنسا وبذور البكتول المحلية بواسطة قياس كروماتوغرافي الغاز-مطياف الكتلة GC-MS و مقارنتها بالدراسات السابقة. تم زراعة بذور الهيبيروك و بذور الباكتول المحلية في حقل كردا رشا، كلية الزراعة ، جامعة صلاح الدين – اربيل, تم تخزين البذور لتحليلها لاحقاً. أظهرت نتائج GC-MS أن أكثر المكونات الكيميائية النباتية الموجودة كانت E و E و 2 و Decadienal-4 (22.3٪ لزيوت بذور Hybrirock و 15.64٪ لبذور Pactol) وHexanal (18.37 لبذور Hybrirock و 8.72٪ لزيوت بذور Pactol)، يليه Undecenal-2 (7.66٪ لبذور الهجين و 14.27٪ لزيوت بذور البكتول). أوضحت دراستنا أن كلا من الأنماط الجينية التي تمت دراستها من نفس الأنواع تحتوي على مواد كيميائية مهمة مختلفة وأحماض دهنية غير مشبعة مفيدة، إلا أن الجلوكوزينولات كانت أعلى بشكل ملحوظ في بذور البكتول بالمقارنة مع بذور الهيربروك، مما يجعل بذور البكتول أقل قابلية للأكل وأكثر ملاءمة كمبيدات للديدان أو كمصدر للوقود الحيوي. في حين أن بذور الهيبيروك ذات المحتوى العالي من الأحماض الدهنية وأقل جلوكوزينولات تمتلك ميزات أكثر للأكل، مما يجعلها اكثر ملاءمة كمنتجات غذائية و طبية.

INTRODUCTION

Searching for renewable energy and other raw chemicals from nature has become a trend among nations nowadays. Scientists have explored Biodiesel as fossil-based fuel substituents, describing it as a renewable energy.

Expensiveness of the biodiesel production is possessing great obstacles nowadays. Rapeseeds, brassica family known to have high amount of erusic acids and glucosinolates (GLS), whose hydrolytic products and other anti-nutritional substances are harmful, especially to non-ruminant animals thus not widely as an animal feed [1]. In that regard the attention toward the possible industrial use of

Brassica seed oils have increased by many developed countries, as their efficiency confirmed by previous works [2].

The rapeseed oils represent the third edible oils in the world and better choice for developing a genotype of seed oils with maximum oil efficiency [3]. By virtue of high PUFA contents like (%60 ca.) oleic acids and Linolenic acid contemplated to round (10% ca.). Food with greater oleic acid is more appreciated by the consumers with its positive health impact. Also have been used as medicinal product because of its antioxidant activity. Traditionally role of rape seed oils as anti-scurvy, anti-inflammatory of bladder, diuretic and anti-goat has been known by many nations. In addition, recent studies showed its effectivity for other usages such as greases, lubricant oils and bio-fuel raised cultivator's intention toward more rapeseed planting [4]. In addition, two undesired constituents of rapeseed oils erusic acid and glucosinolates possess difficulties for cultivators who still working harder on developing better quality brassica Oils with a content of less than 2% erusic acid and not more than 30 µm glucosinolate compounds [5]. It's proven that Gas Chromatography coupled with Mass Spectrometry (GC-MS) is very precise, less time consuming and cheaper than the traditional extraction methods [6].

Hybrirock is a genotype of brassica napus L. that has been cultivated for years in France due to its edibility and beneficial nutritional support. Efficiency of variants, generated by certain concentrations of fertilizers has also confirmed impact of higher concentrations; in terms of dosages and prices of yields (NPK+Mn chelate 30% and NPK+Mg SO4 35%). K [7].

Pactol genotype of brassica napus is an important local gene in Iraq and Middle East. Previously many research studies done in these regions in purpose of the increasing yield and improving its nutritional content [8].

GLS are secondary metabolites accommodated Sulphur chemicals mainly brought by a vegetable plant called Brassica. Researchers showed that toxic hydrolytic products formed during interacting myrosinase (thioglucoside glucohydrolase 3.2.3.1) enzyme and GLS in a reaction of water involvement. A byproduct will be goitrogenic and potentially hepatoxic compounds, such as isothiocyanates and thiocyanates. Researchers exhibited that GLS-containing Brassica vegetables may have anticarcinogenic effects.

Although previous studies evidenced ant carcinogenic role of GLS-containing Brassica plants but industrial interest is toward developing of a genotype with minimum percentage of glucosinolates because of its undesired bitter taste reducing its edibility as commercial food product [9].

Our study aims to compare GC-MS inquiry of the phytochemical constituents of two Brassica napus genotypes (Hybrirock and pactol seeds),

implementing additional knowledge on those genotypes which may aid plant breeders in their coup of designing brassica seed genotype with maximum seed oil efficiency.

MATERIALS AND METHODS

Plant cultivation and Sample preparation

The hybrirock seeds of Brassica napus L. were purchased from France and pactol seeds purchased locally in Erbil. The identification and authentication done by department of field crop/ college of agricultural engineering and the seeds were cultivated during the winter growing season at Grdarasha Research Field / College of Agriculture / University of Salahaddin / Erbil. Plant seeds freshly collected and air dry temperature of 40 °C for later analysis. Crushing of dried seeds performed using mortal and pestle. The crushed seed materials transferred into the Soxhlet extractor and n-hexane was added as a solvent, then the process was let to run for 3 hr. the essential oils were collected in tightened vials and stored in a refrigerator at 10 °C [10].

Phytochemical analysis

Analysis of Each oil extracts was done by using Shimadzu Model QP-2010 GC coupled with MS. The

GC equipped with HP-5 MS (5% phenylmethylsiloxane), capillary column (30 m × 0.25 mm i.d., film thickness 0.25µm). in the temperature program 60 °C (2') to 250 °C for 10 minutes with rate of 20 °C /min, helium flow rate 1.61ml/minute. The ion source maintained at 250 °C with electron energy 70 eV. The oil extract samples added into the methanol and then 1μl injected in the column. Based on the Wiley library, the unknown component was recognized based on the comparison of their mass spectrum with the spectrum of the known components. Chemical identifications including names, chemical structure and molecular weight of sample contents were eventually obtained. In contrast with [11].

RESULTS

According to the obtained data shown in Table 1, Phytochemicals detected from seed oils were twenty seven and twenty five for Hybrirock seed and Pactol genotypes respectively. The results showed a difference in the number of compounds in one species of two genotypes. chromatogram of Hybrirock seed n-Hexan extract is represented in (Table 1), showing twenty-seven peaks which means 27 phytochemicals comprise 99.98% of essential oils of hyprirock seed oils. Aldehydes were predominant organic groups including 2,4-Decadienal, (E, E)- with the highest peak percentage of total phytochemicals (22.26 %), followed by Hexanal (18.37 %) and 2-Undecenal (7.66 %) were the main components. While ketons were second most abundant chemical groups mainly composed by 4-Nonanone, 7-ethyl- (6.53%) and the main fatty acids were 9-Octadecenoic acid (oleic acid), Pentadecanoic acid (Palmitic acid) and octadecanoic acid (stearic acid) with their percentages (6.62%), (1.43%) and (0.73%) respectively. Similarly the results of GC-MS of local pactol seed oils revealed that Aldehydes were highest chemical constituents with 2,4-Decadienal, (E, E)-(15.64%), 2-Undecenal(14.27%),2-Decenal, (E)-(13.61%), Hexanal(8.72%).The other chemicals found to be present in pactol seeds were Cyclohexanone, 2-ethyl, 1H-Purin-6-amine, [(2-fluorophenyl) methyl]-, 6-Azabicyclo [3.2.1] octane, Hexanoic acid, 2-hexenyl ester, (E)- Various terpenes , esters and other glucosinolates shown in Table 1.

As shown in (figure 1), which represents the chemical compounds of n-Hexan extraction essential oil from plant seeds of Hybrirock genotype. The highest peak value was recorded for 2,4-Decadienal at retention time 8.313 min. and the lowest peak value was for 4,8,12-Trimethyltridecan-4-olide at retention time 9.730 min.

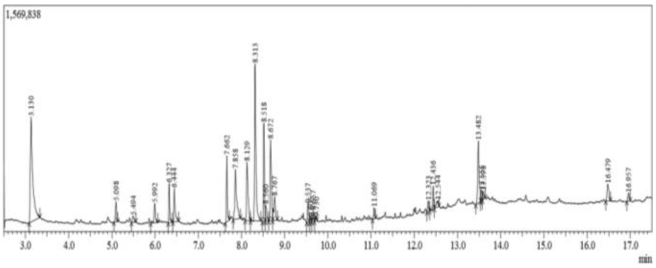

Figure 1. GC-MS chromatogram of n-Hexan extract of hybrirock seeds of brassica naps.

The Chromatogram from GC-MS results of pactol seed extracts showed twenty five peaks with the maximum peak recorded for 2,4-Decadienal, (E, E)- and the minimum peak value recorded for 12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en, (Table 1).

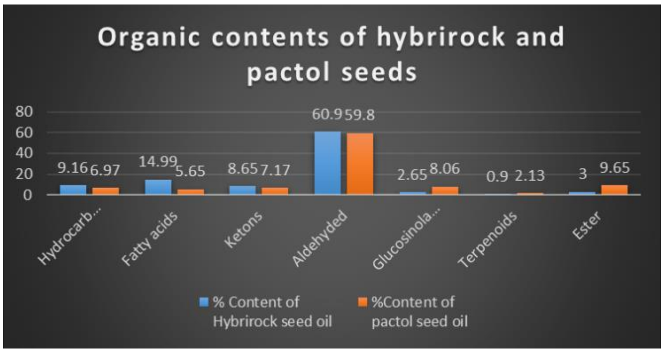

Figure 2. Organic class contents of Hybrirock and pactol seeds of brassica naps.

The average percentages of the total phytochemical contents referred as essential oil components and other compounds. In total, 27 phytochemicals compounds of Hybrirock seed oils can be grouped into seven main chemical classes, the relative intensity of organic compounds for hybrirock seeds showed hydrocarbons (9.16%), fatty acids (8.82%), Ketons (8.65%), Ester (3%), Aldehydes (60.9) as well as carboxylic acids (6.22%) and glucosinolates (3.55%), were identified successively (Table 2).

Table 1. Identified phytochemicals using MS coupled to GC of French Hybrirock and pactol genotypes of Brussica napus L.

Peak #	Hybrirock Seed		Pactol Seed		Molecular formula of detected chemicals
	R. Time	Area %	R. Time	Area %	
1	3.130	**18.37**	3.347	**8.72**	$C_6H_{12}O$
2	5.098	1.73	5.197	2.04	$C_6H_{12}O_2$
3	5.494	0.83	NF	NF	$C_7H_{10}O$
4	5.992	2.01	NF	NF	C_8H14O
5	6.327	2.65	NF	NF	$C_7H_{14}O$
6	6.444	3.06	6.126	4.88	$C_9H_{18}O$
7	7.662	4.49	NF	NF	$C_{10}H_{19}ClO$
8	7.858	**6.71**	6.981	**13.61**	$C_{10}H_{18}O$
9	8.129	6.52	7.134	5.00	$C_{10}H_{16}O$
10	8.313	**15.74**	7.239	**10.64**	$C_{10}H_{16}O$
11	8.518	6.53	NF	NF	$C_{11}H_{22}O$
12	8.560	1.75	NF	NF	$C_{14}H_{24}O$
13	8.672	**7.66**	7.434	**14.27**	$C_{11}H_{20}O$
14	8.767	2.82	NF	NF	C_6H_8O
15	9.537	1.80	NF	NF	$C_{14}H_{20}O_2$
16	9.605	0.50	NF	NF	$CH_3(CH_2)_{17}SiCl_3$
17	9.657	0.90	7.959	1.19	$C_{20}H_{42}$
18	NF	NF	8.729	0.94	$C_{20}H_{42}$
19	9.730	0.47	NF	NF	$C_{16}H_{30}O_2$
20	11.0697	0.79	NF	NF	$C_{22}H_{46}$
21	12.323	0.94	NF	NF	$C_{32}H_{66}$
22	12.436	1.48	9.576	1.23	$C_{15}H_{30}O_2$
23	12.544	1.01	NF	NF	$C_{22}H_{46}$
24	13.482	6.62	10.381	2.41	$C_{18}H_{34}O_2$
25	13.555	0.88	NF	NF	$C_{32}H_{66}$
26	13.598	0.72	NF	NF	$C_{18}H_{36}O_2$
27	16.479	2.15	NF	NF	$C_{19}H_{38}O_4$
28	16.957	0.85	NF	NF	$C_{16}H_{22}O_4$
29	NF	NF	5.419	0.74	$C_8H_{14}O$
30	NF	NF	5.828	2.40	$C_{12}H_{22}O_2$
31	NF	NF	6.050	2.57	$C_8H_{16}O$
32	NF	NF	6.462	0.79	$C_9H_{16}O$
33	NF	NF	6.696	1.20	$C_{10}H_{20}O$
34	NF	NF	6.865	**3.54**	$C_8H_{15}NO$
35	NF	NF	6.910	1.53	$C_{10}H_{16}O$
36	NF	NF	7.349	8.71	$C_{19}H_{36}O_3$
37	NF	NF	7.486	3.45	C7H13N
38	NF	NF	7.890	0.76	$C_9H_{14}O_3$
39	NF	NF	7.922	1.23	$C_{17}H_{28}O_3$
40	NF	NF	7.992	1.06	$C_8H_{12}O_3$
41	NF	NF	10.470	**6.36**	$C_{12}H_{10}FN_5$
42	NF	NF	11.363	0.73	$C_{14}H_{24}O_3$
		99.98%		100.00%	Total

Table 2. Organic class Compositions of seed essential oils of Hybrirock and pactol genotypes.

No.	Organic class	Chemical compounds of Hybrirock seeds	Content % of Hybrirock	Chemical compounds of Pactol seed	Content % of Pactol
1	Hydrocarbons	1,13-Tetradecadien-3-one, 1,4-Epoxycyclohex-2-ene, Silane, trichlorooctadecyl- (CAS), -, Docosane ,Dotriacontane	9.16	12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en, , Bicyclo[3.1.1]heptan-2-one, 3,6,6-trimethyl-,6-Azabicyclo[3.2.1]octane, , trideuteriomethyl 10-epoxy-7-,7-, 2-methyl- ethyl,	6.94
2	Fatty acids	Pentadecanoic acid (CAS) Hexanoic acid,P almitic acid, 9-Octadecenoic acid (Z)- (CAS) oleic acid octadecanoic acid, (stearic acid)	14.67	Pentadecanoic acid (CAS) Hexanoic acid, 9-Octadecenoic acid (Z)- (CAS) oleic acid,Erusic acid, Arachidic acid	6.14
3	Ketones	2-Hexen-4-ol, 5-methyl.., 4-Nonanone, 7-ethyl-,, 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethyl), p-Benzoquinone	8.65	Cyclohexanone, 2-ethyl 6-Methyl-hept-2-en-4-ol,7-Oxabicyclo[4.1.0]heptane, 2-methyl-	7.17
4	Aldehyde	Hexanal (CAS), 2,4-Heptadienal, (E,E)- (CAS), 2 OCTENAL., Nonanal (CAS), 2-Decenal, (E)-, 2,4-Decadienal, (E,E)- (CAS), 2-Undecenal	60.9	Hexanal, Octanal, Nonanal ,2-Nonenal, Decenal, 2-Decenal, 2,4-Decadienal, (E,E, 2-Undecenal	59.85
5	Glucosinolate	2-Hexen-4-ol, 5-methyl-,	2.65	1H-Purin-6-amine, [(2-fluorophenyl)methyl, ,1,2-Butanediol1-(2-furyl)-2-methyl	8.06
6	Terpenoids	Hexadecane, 2,6,10,14-tetramethyl	0.90	Hexadecane, 2,6,10,14-tetramethyl	2.13
7	Ester	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester,1,2-Benzenedicarboxylic acid, mono(dimethylcyclohexyl) ester	3	Hexadecanoic acid, 2-hexenyl ester, Octadecanoic acid, 2-oxo-, methyl ester	9.65
Total			99.98%		100 %

The glucosinolates and esters were notably had lower relative abundance in Hybrirock seed oils (3.55%) in compare with pactol Seed oils (9.25%). While the relative intensity of fatty

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acids and carboxylic acids were notably higher in Hybrirock seeds in compare with pactol seed oils. Glucosinolates and esters were the second most abundant chemical group in pactol seed oils unlike french Hybrirok seed oil constituents, mainly including 1H-Purin-6-amine, [(2-fluorophenyl) methyl] - (6.38%), Cyclohexanone, 2-ethyl-(3.54), 6-Azabicyclo [3.2.1] octane (3.45%), also notably higher percentage of esters compounds were present in pactol oil extractions mainly were Octadecanoic acid, 2-oxo-, methyl ester (8.71%) and Hexanoic acid, 2-hexenyl ester (2.40%) as shown in (Table 1).

DISCUSSION S

The present study detected the fatty acids (oleic acid), essential oil (sesquiterpene and monoterpene hydrocarbons) and other chemical compositions of Hybrirock and pactol seeds of the brassica napus by GC-MS. The majority of these compounds were known to be very valuable commercially and medicinally [12]. In the case of hybrirock seeds, the aldehydes and ketons were found to be amongst the highest contributors (see Table 1). The seeds oil was largely represented by E-E 2.4, decadenial in both seed oil genotypes and oil constituents inquired based on percentages. Pactol seed oils contains abundant quantities of several fatty acids and their derivatives (a total of about 30.54%), also Oxygenated monoterpene hydrocarbons represented the main fraction of the seed oil (52.61%) followed by oxygenated susquiterpens (4.91%) and oxygenated diterpenes (2.13%). The remaining percentage (9.81%) was recorded for other secondary metabolites, each of which may possess potential benefit such as pharmaceutical agent. While Hybrirock seed oils showed higher relative abundance for fatty acids and their derivatives (46.75%), followed by oxygenated monoterpenes and Oxygenated susquiterpens. (table 1). The present inquiry conceded by [13].

Phytochemical analysis of both seed oils recorded highest percentage content for (E,E)-2,4-Decadienal which followed by 2-Undecenal, which are monoterpenes and aromatic substance found in many foods such as butter, cooked beef, fish, potato chips, roasted peanut, buckwheat and wheat bread crumb. it smells of deep fat flavor in isolated form which is characteristic of chicken aroma (at 10ppm).Uptake of 2,4-decadienal caused decreased body weights and increased incidences of forestomach lesions in the 3-month studies in rats and mic. Studies have settled the nematicidal potentials of (E,E)-2,4-decadienal and (E)-2-decenal [14].

Another most common chemicals found to be present in two tested brassica genotypes was Hexanal, its level was notably higher in Hybrirock seeds than in pactol seed oils. Its potent odorants that contribute to the crust and crumb flavor of wheat and rye breads [15]. Previous works have documented that in virtue of its interference capabilities to cytoplasmic membrane of microorganisms, the hexanal contemplated as antimicrobial agent [16].

A research done by [17] explained that n-Hexanal (hereafter called hexanal) is present in volatile organic chemicals, used as flavoring additives in both conventional and e-cigarettes.

2-Undecenal, also present in both seed oils but its peak percentage significantly higher in pactol seeds (14.2%) than hybrirock seeds (7.66%). A study by [18] concluded that trans-2-undecenal is mainly formed due to the oxidation of triolein while heating. Also it can be found naturally in coriander, fresh red pepper and watermelon which can be used as flavoring agent as reported by [19].

The results of the fatty acid composition of hybrirock seeds of brassica napus L. presented in (Table 1). The inquiry showed that pre-dominant fatty acid was Oleic acid (C18: 1 n-6), this was in accordance with a study by [20] performed on six different rape-seed genotypes.

The fatty acids percentages and other compounds derived from fatty acid in hybrirock seed oils were observed to surpassed its content in pactol seed oils. Same results found by [21] using Hp-LC. Previously shown that Monounsaturated fat consumption has been associated with decreased low-density lipoprotein (LDL) cholesterol, and debatably, increased high-density lipoprotein (HDL) cholesterol [22].

Glucosinolates were notably had lower relative intensity in hybrirock (3.55%) seed oils in compare with seed oils of pactol (9.25%). Which make hybrirock seeds oils to be more suitable to be harvested as animal feed and also can be used in food and medical industries. Because of good edibility quality and less bitterness than pactol seed oils. Presence of GLS in pactol seeds previously reported by [23]

Phytochemical compounds of pactol seeds oils contained several hydrocarbons, esters and aldehydes (Table 1) which were found to be absent in hybrirock seed oils such as 1H-Purin-6-amine, [(2-fluorophenyl) methyl] - Which known to have many biological and antimicrobial roles[24]. In contrast, GC-MS result of hybrirock seed oils showed several aldehydes, ketones and fatty acids, like Silane (trichlorooctadecyl) participates in manufacturing of compounds containing silicone. (Table 1), which were not present in Pactol seeds. Scientists explained those chemical constituent differences of two seeds of same species are due to their genetic factors, geographical variations, chemo typic diversity, plant maturation stage, nutritional status of the plant and environmental conditions (25). Also the biotic and abiotic stresses possess impact on secondary metabolites like terpenes, phenols and fatty acids which are clearly explained by many researchers (26). The differences in essential oil compositions of hybrirock and pactol seed oils may be attributed to one or any combination of these elements.

CONCLUSIONS

Keeping up with vast growing pharmaceutical industry is very challenging among drug companies and designing new active agent with minimum side damage made this task even harder. Extraction of medicinal plants with less side effects than drugs can be inquired as substitute of these chemicals for vast potential usage in fields of medicine, cosmetics, edible material and biodiesel production, As the two tested genotype seeds have shown sufficient hydrocarbon content which can be used as a substitute of petrol hydrocarbon. as there is increased tilt toward usage of secondary product as natural additives for biofuel production.

The present inquiry delivered the fact that both cultivated genotypes of B.napus exhibited variable phytochemicals and beneficial polyunsaturated fatty acids at different percentages. Glucosinolates were noticeably higher in pactol seeds in compare with hybrirock seeds, making pactol seeds less edible because of GLS bitterness taste and their toxic effects while hybrirock seeds with higher fatty acid contents and lower glucosinolates possess more edibility features to be used as food products and medical agents.

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