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RESEARCH ARTICLE

GLUCOSINOLATES IN SOME BRASSICA SPECIES AS SOURCES OF BIOACTIVE COMPOUNDS AGAINST ROOT KNOT NEMATODES.

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Abstract

Brassica species are sources of bioactive compounds with several biological properties including biocidal activity against various soil borne pathogens and pests such as parasitic nematodes. Isothiocyanates derived from corresponding glucosinolates are major bioactive compounds responsible for this activity. In this study, glucosinolate content of red and white radish (*Raphanus sativus* L.), oilseed rape (*Brassica napus* L.), turnip (*Brassica rapa* L.) and Arugula (*Eruca sativa* L.) that were previously assessed for their host suitability level of root-knot nematodes (*Meloidogyne arenaria* and *Meloidogyne incognita)* were determined to understand the relationship between glucosinolate content and host-suitability level of these crops. The highest glucosinolate content was in radish. Turnip revealed lower levels compared to radish. However, the lowest glucosinolate content was determined in arugula and oilseed rape. Together with previous findings demonstrating host-suitability levels, the effect of glucosinolates on biocidal potential of *Brassicaceae* plants to fight against root-knot nematodes were evaluated.

Introduction:-

The incorporation of plants containing specific biologically active compounds into soil against soil-borne pests is a natural plant protection approach. The *Brassicaceae* plants synthesize glucosinolates (GSL) that are sources of bioactive compounds such as isothiocyanates with several biological properties including biocidal activity. They are classified as aliphatics, aromatics or indoles having different properties and functions. Genetic factors determine the glucosinolate profile of plants, therefore the glucosinolates produced by a plant may vary. Glucosinolate content, however, is under the influence of environmental factors as well as stress factors during the growth period as reviewed by Sarıkamış 2009. Although present in the entire plant, the amount of glucosinolates is variable at different plant parts and plant growth stages (Brown *et al.*, 2003).

Glucosinolates are hydrolyzed by endogenous myrosinases to produce an array of compounds including isothiocyanates, nitriles and indoles. Among these compounds isothiocyanates are associated with several biological activities including the suppression of soil borne pests (Lin *et al.*, 2000). Benzyl isothiocyanate derived from the

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hydrolysis of glucotropaeolin and 2-phenylethyl isothiocyanate has been shown to have high levels of antibacterial activity (Jang *et al.,* 2010) and toxicity to several soil pathogens including nematodes and fungi (Jensen *et al.,* 2010). Root-knot nematodes are plant parasitic nematodes that cause high losses in plants by reducing its quality and quantity. Chemical nematicides including soil fumigants have effectively controlled nematodes (Nyczepir and Thomas 2009). However, in recent years, restrictions on the use of these chemicals due to adverse effect on environment and human health have increased interest in non-chemical alternatives (Melakeberhan *et al.,* 2006; Lopez-Perez *et al.,* 2010; Edwards and Ploeg 2014). *Brassicaceae* plants have been reported to possess a great potential to reduce *Meloidogyne* spp. population in the means of biofumigation (Curto *et al.,* 2005; Monfort *et al.,* 2007). In tomato, Brazilian wild mustard tissues amended in soil revealed nematicidial activity against *Meloidogyne incognita* (Oliveira *et al.,* 2011). Soil incorporation of broccoli was reported to reduce root galling on tomato by 36% (Lopez-Perez *et al.,* 2010). Monfort *et al.,* (2007) reported that incorporation of selected Brassica species as green manure lowered nematode populations and root damage caused by *M. incognita* infection more than non-Brassica species. However, nematode control activity was found variable among *Brassica* species depending on host suitability level (Morra and Kirkegaard 2002; Zasada *et al.,* 2003; Hartz *et al.,* 2005; Monfort *et al.,* 2007; Ntalli and Caboni 2012; Avato *et al.,* 2013). Nematode-suppressive activity of Brassica species has met with variable results and this variability can be explained by some major factors including glucosinolate profile in plant tissues (Zasada *et al.,* 2010). Soil-borne pests and diseases suppression by product of glucosinolate hydrolysis (most commonly isothiocyanates) released from incorporated plant tissues related to identities and concentrations of glucosinolates in plant tissue (Morra and Kirkegaard 2002). Therefore, it is crucial to know glucosinolate content of the plants to be used for biofumigation.

A good biofumigation crop for nematode management should be a poor host for target nematode species (Edwards and Ploeg 2014) and also have high glucosinolate production (Monfort *et al.,* 2007). In our previous study, we investigated 40 genotypes from 15 different *Brassica* species initially to assess their host suitability level of root-knot nematodes (*M. arenaria* and *M. incognita*) and 12 genotypes were found to be as poor host with low potential of nematode multiplication as cover crop for biofumigation (Aydınlı and Mennan 2016). The objective of our study was to determine the glucosinolates of the selected brassica species that were known as poor hosts for *M. arenaria* and *M. incognita,* thus to have an idea of the relation between glucosinolates and biofumigation effect of the Brassica plants.

Materials And Method:-

Plant material:-

Brassicaeae spp. including red and white radish (*Raphanus sativus* L.), oilseed rape (*Brassica napus* L.), turnip (*Brassica rapa* L.) and Arugula (*Eruca sativa* L.) previously tested for host suitability to root-knot nematodes (Aydınlı and Mennan, 2016) were used for the analysis of glucosinolates. These species were selected among the poor hosts for *M. arenaria* and *M. incognita* according to Aydınlı and Mennan (2016). Egg masses index, gal index and % RS (Relative Susceptibility= total nematode number on tested plant / total nematode number on susceptible control plant x 100) that are used as criteria to evaluate host status of plant are given for each species in Table 1.

Analysis of glucosinolates:-

Extraction of glucosinolates were performed on lyophilized leaf tissue using 70% (v/v) methanol, desulfated using Type H-1 Sulfatase from *Helix pomatia* (Sigma®) using DEAE Sephadex™ column, collected in vials and analyzed by HPLC (Shimadzu®) at Ankara University, Faculty of Agriculture, Department of Horticulture. Desulfoglucosinolates of each species were analyzed and separated by HPLC- UV detection using Waters Spherisorb 5µM ODS 2, 4.6x250mm analytical cartridge with a gradient program of 99% water and 1% acetonitrile as 1ml/min for 24 min (Sarıkamış et al. 2006) at a wavelength of 229 nm. Sinigrin from horseradish (Sigma®) or glucotropaeolin (Applichem®) which is not synthesized by the plant itself was used as the internal standard at a concentration of 16mM for the quantification of the peaks and given as $\mu mol g^{-1}$ dry weight. A correction factor during calculation of each compound is provided by Brown *et al.,* 2003.

The peaks were identified using pure standards glucoraphenin (4-methylsulsulfinyl-3-butenyl), gluconapin (3-butenyl), progoitrin (2-hydroxy-3-butenyl), glucoerucin (4-methylthiobutyl) purchased from PhytoLab GmbH&Co., sinigrin (2-propenyl glucosinolate) and glucotropaeolin (benzyl glucosinolate). The standards were desulfated prior to use and run in each sequence as external standards together with plant extracts.

Statistical Analysis:-
The analysis for each species was performed as three replicates. The glucosinolate contents were determined as mean ± standard error (SE) of the mean.

Results:-

Glucosinolates in Red and White Radish *(Raphanus sativus* L.*)*
The major aliphatic glucosinolate in red and white radish samples was glucoraphenin (4-methylsulfinyl-3-butenyl) as the major glucosinolate comprising 54% of the total glucosinolates. In addition to glucoraphenin, glucoraphasetin (4-methylthio-3-butenyl) was also present in radish comprising 30% of the total glucosinolates. Glucobrassicin (3-indolylmethyl) of indoles comprised 9.4% of the total glucosinolates (Fig.1).

Quantification of individual glucosinolates revealed that while glucoraphenin content was 21.18±2.5 µmolg^{-1}DW in red radish and 17.89±1.33 µmolg^{-1}DW in white radish, glucorapasetin content was 6.52±0.69 µmolg^{-1}DW and 11.79±0.48 µmolg^{-1}DW in red and white radish, respectively. In terms of indole glucosinolates, glucobrassicin content was 1.61±0.21 µmolg^{-1}DW and 1.21±0.15 µmolg^{-1}DW for red and white radish, respectively (Table 2). Comparing the results with RS%, the genotypes with high glucosinolate content revealed less than 10% RS values for *M. arenaria* and *M. incognita* suggesting potential use for biofumigation approaches.

Glucosinolates in turnip *(Brassica rapa* L.*)*
Aliphatic glucosinolates in turnip PI352811 (*Brassica rapa* L.) were glucobrassicanapin (4-pentenyl) accounting for 30.8% of the total glucosinolates followed by progoitrin (2-hydroxy-3-butenyl) (18.7%) and gluconapin (3-butenyl) (14.7%). In terms of indole glucosinolates, glucobrassicin (3-indolylmethyl) (8.8%), and 4-hydroxyglucobrassicin (4-hydroxy-3-inolylmethyl) (6.1%) were determined (Fig. 2).

Quantification of each compound suggested that glucobrassicapin content in turnip was 3.22±0.19 µmolg^{-1}DW, progoitrin content was 2.06±0.11, µmolg^{-1}DW, gluconapin content was 2.0±0.05 µmolg^{-1}DW, glucobrassicin was 0.47±0.16 µmolg^{-1}DW and 4-hydroxyglucobrassicin was 0.19±0.08 (Table 2). Gluconapin and glucobrassicanapin are the aliphatic glucosinolates. While the sum of the relative proportions of aliphatics was around 64%, two indoles (4-hydroxyglucobrassicin and glucobrassicin) was around 15% of the total glucosinolates. The amount of glucosinolates in PI 352811 turnip genotype was low compared to red and white radish genotypes.

Glucosinolates in oilseed rape *(Brassica napus* L.*)*
Glucosinolate profiling of oilseed rape (B13) revealed progoitrin (2-hydroxy-3-butenyl) and sinigrin (2-propenyl) of the aliphatic group accounting for 23.6 and 22.9%, respectively. In terms of indoles, glucobrassicin (3-indolylmethyl) (39.6%) and 4-hydroxyglucobrassicin (4-hydroxy-3-indolyl) (13.9%) were found in oilseed rape (Fig. 3). These findings suggest that indole glucosinolate glucobrassicin was the predominant glucosinolate in oilseed rape.

Quantification of the results using glucotrapeolin as the internal standard revealed that glucobrassicin content was 0.57±0.03 µmolg^{-1}DW, 4-hydroxyglucobrassicin content was 0.20±0.02 µmolg^{-1}DW, progoitrin was 0.34±0.04 µmolg^{-1}DW and sinigrin was 0.33±0.08 µmolg^{-1}DW in oilseed rape (Table 2). Overall, total and individual glucosinolate contents were very low in oilseed rape compared to other brassica species used in the present study.

Glucosinolates in Arugula *(Eruca sativa* L.*)*
Glucosativin (4-mercaptobutyl glucosinolate) was determined as the major glucosinolate in Arugula (*E. sativa* cv. Istanbul) (Fig. 4). Quantification of the results using internal standards suggested that glucosativin content was 1.81±0.14 µmolg^{-1}DW in the Arugula leaf tissue analyzed (Table 2). This compound is convertes to 4-mercaptobutyl isothiocyanate (sativin), a volatile and pungent metabolite probably responsible for the typical flavor of Arugula.

Discussion:-

Aliphatic and indole glucosinolates in red and white radish, turnip, oilseed rape and arugula revealed as poor hosts for *M. arenaria* and *M. incognita* (Aydınlı and Mennan 2016) were determined in the study. According to the findings, the highest level of glucosinolates was quantified in radish containing glucoraphenin as the predominant glucosinolate followed by turnip containing glucobrassicanapin, progoitrin and gluconapin as the major glucosinolates. Aragula revealed lower levels compared to radish with glucosativin as the predominating glucosinolate. The lowest glucosinolate content was determined in oilseed rape determined as progoitrin and sinigrin at very low concentrations.

Potter *et al.,* (1998) reported that leaf tissues of high glucobrassicanapin and progoitrin containing *B. rapa* significantly reduced populations of root lesion nematode *Pratylenchus neglectus* (66%) when amended in soil. Strong nematicidal activity of isothiocyanates derived from sinigrin (2-propenyl isothiocyanate) was reported in vitro on juveniles of *H. schachtii* after 24 hours at 0.5% concentration (Lazzeri *et al.,* 1993). Lazzeri *et al.,* (2004) demonstrated stronger activity of the isothiocyanate on *M. incognita* in vitro. Aside from plant-parasitic nematodes, sinigrin isothiocyanate showed also high biocidal activity on other soil-borne pathogens (Mayton *et al.,* 1996)

Glucosativin (4-mercaptobutyl), glucoerucin (4-methylthiobutyl) and glucoraphanin (4-methylsulfinylbutyl) are the most prominent glucosinolates within the leaf tissue of *Eruca* species (Pasini *et al.,* 2011; Villatoro-Pulido *et al.,* 2013). Bell *et al.,* (2015) tested 25 *E. sativa* accession for glucosinolate and flavonol content, and glucosativin were identified in all accessions with 91.3% of the total glucosinolates. Aissani *et al.,* (2015) showed that the soil amendment of fresh Arugula in tomato decreased the nematode infection (*M. incognita*) in a dose-response manner (EC50 = 20.03 mg/g) improved plant growth. Our results also in agreement with this research reporting *E. sativa* as a promising plant in intercropping strategies for tomato against root-knot nematodes.

Ngala *et al.,* (2015) examined the biofumigation potential of *B. juncea*, *E. sativa* and *R. sativus* against potato cyst nematode *Globodera pallida* suggesting that while *R. sativus* reduced the viability of *G. pallida* encysted eggs, *B. juncea* reduced the viability in summer, but not when grown in winter conditions. Viability of *G. pallida* in with *E. sativa* did not differ from the untreated fallow in treated plots, suggesting the low concentrations of isothiocyanate source of glucosinolates in *E. sativa.*

Brassica crops are important for biofumigation as an effective way instead of synthetic chemicals to control soil borne pests and diseases. This is mainly attributed to glucosinolates which are converted into isothiocyanates on hydrolysis by the enzyme myrosinase. Isothiocyanates can reduce the activity of pathogens and pests in the soil (Ntalli and Caboni, 2017). The current study revealed that among different brassica species, radish (red and white cultivars) had the highest glucosinolates. Aliphatic glucosinolates were the major compounds followed by indoles at low levels in radish. Glucosinolates in other brassica species were much lower compared to radish. Both aliphatics and indoles were almost equal in turnip and oilseed rape. Glucosativin (4-mercaptobutyl glucosinolates), the precursor of sativin (4-mercaptobutyl isothiocyanate) was identified at low concentrations in Arugula. Therefore, radishes in this study may have a more biofumigation potential than other brassica plants if utilized as a green manure amendment. In order to increase the success of biofumigation, non-host or poor host species for target nematode species or population should be grown especially when soil temperature is suitable for nematode activity (Stirling and Stirling 2003; Pattison *et al.,* 2006; Avato *et al.,* 2013). Otherwise, nematode population increases in the soil during cultivation before the incorporation of biofumigant plants into the soil. According to the present findings non-host or poor host brassica with high glucosinolate content converting the majority of glucosinolates into isothiocyanates should be preferred to succeed in biofumigation approaches.

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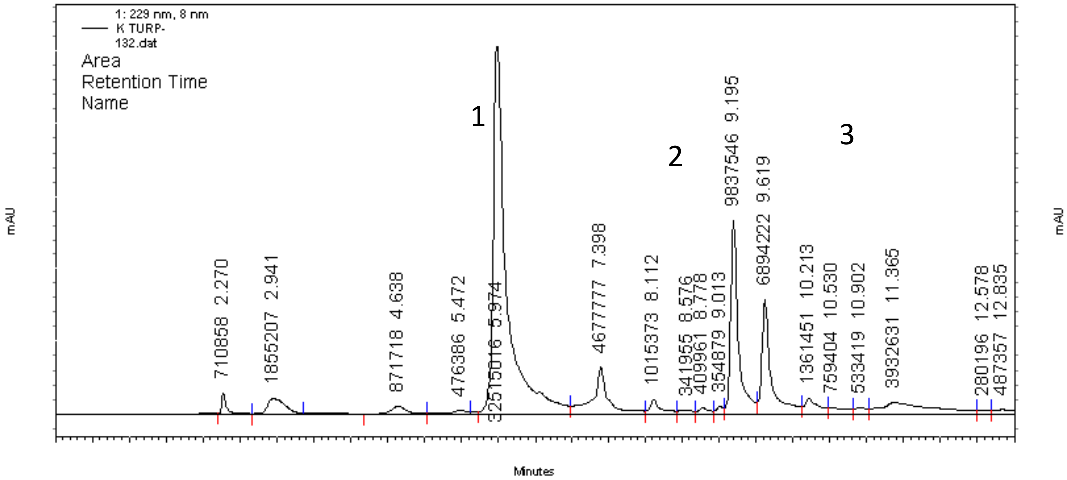

Figure 1:- The Glucosinolate profile of radish (*Raphanus sativus* L.) *1:Glucoraphenin; 2:Glucoraphasetin; 3:Glucobrassicin*

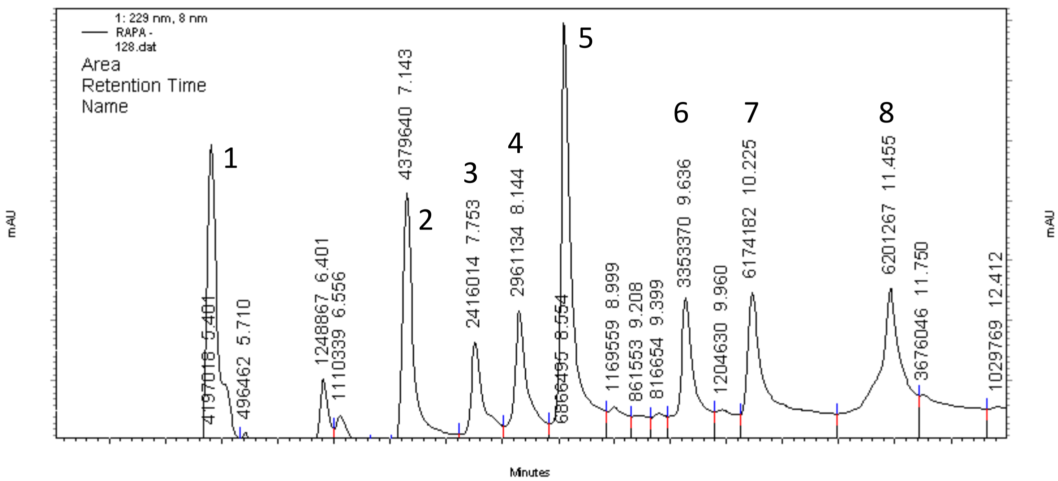

Figure 2:- The Glucosinolate profile of turnip (*Brassica rapa* L. PI352811) *1:Progoitrin;2:Glucoallysin; 3:Gluconapin; 4:4-Hydroxyglucobrassicin; 5:Glucobrassicanapin; 6:Glucobrassicin; 7:4-Methoxy-3-indolylmethyl; 1-Methoxy-3-indolylmethyl*

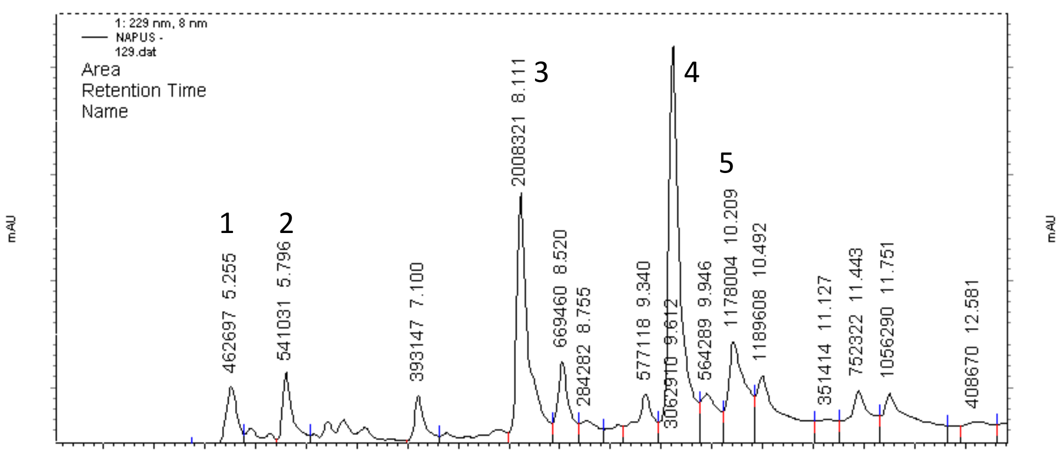

Figure 3:- The Glucosinolate profile of oilseed rape (*Brassica napus* L. B13) *1:Progoitrin; 2:Sinigrin 3:4-Hydroxyglucobrassicin; 4:Glucobrassicin; 5:4-Methoxy-3-indolylmethyl*

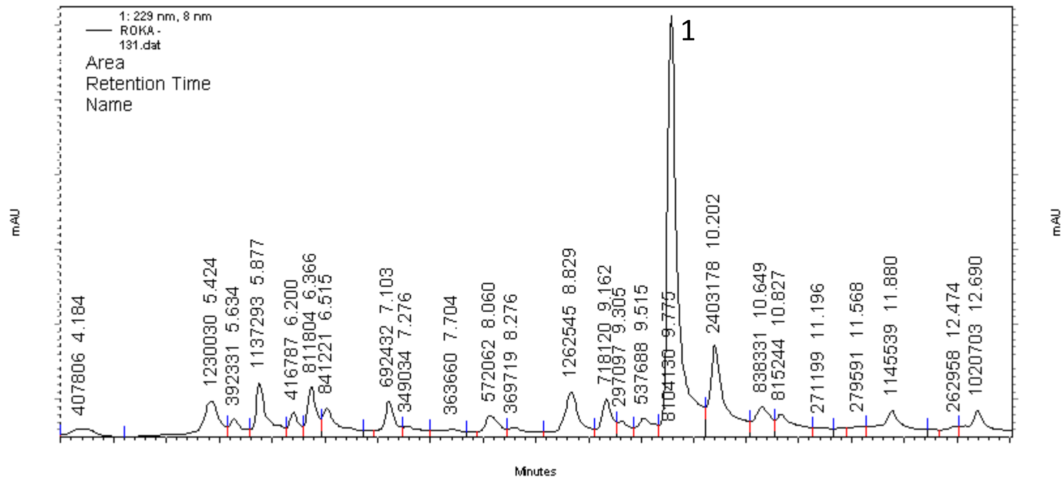

Figure 4:- The Glucosinolate profile of Arugula (*Eruca sativa* L.) *1: Glucosativin*

Table 1:- Host status of *Brassica* species for *Meloidogyne arenaria* and *Meloidogyne incognita*, 60 days after inoculation with 2,000 eggs per plant in a pot experiment conducted in a controlled greenhouse (20±1°C)[1]

Plant Species	Genotype	*M. arenaria*			*M. incognita*		
		EI[1]	GI[2]	RS (%)[3]	EI[1]	GI[2]	RS (%)[3]
Brassica napus (Oilseed rape)	B13	0,00	2,50	11,75	0,00	2,80	9,60
Brassica rapa (Turnip)	PI 352811	0,10	2,10	8,40	0,30	2,00	6,48
Raphanus sativus (Radish)	White	0,30	0,80	6,33	0,00	0,70	0,86
	Red	0,40	0,90	4,79	0,00	1,00	4,07
Eruca sativa (Arugula)	İstanbul	0,20	0,20	3,46	0,00	0,20	5,41

[1] This result was reported by Aydınlı and Mennan (2016).
[2] EI= Egg Masses Index (0-5): 0= no egg masses, 1= 1-2 egg masses, 2= 3-10, 3= 11-30, 4=31-100, 5= more than 100 egg masses (Taylor and Sasser, 1978).
[3] GI= Gall Index (0-5): 0 = no galls, 1 = with a few small galls, 2= <25% roots galled, 3= 25-50%, 4= 50-75%, and 5= >75% of root galled (Kinloch, 1990).
[4] RS= Relative Susceptibility: total nematode number on tested plant / total nematode number on susceptible control plant x 100

Table 2:- Glucosinolate content of brassica species (µmolg^{-1}DW)

Glucosinolates	*Raphanus sativus* (Radish-Red)	*Raphanus sativus* (Radish-White)	*Brassica rapa* (Turnip "PI 352811")	*Brassica napus* (Oilseed rape "B13")	*Eruca sativa* (Arugula cv. Istanbul)
Aliphatics					
Glucoraphenin	21.18±2.5	17.89±1.33	-	-	-
Glucoraphasatin	6.52±0.69	11.79±0.48	-	-	-
Progoitrin	-	-	2.06±0.11	0.34±0.04	-
Sinigrin	-	-	-	0.33±0.08	-
Gluconapin	-	-	2.00±0.05	-	-
Glucobrassicanapin	-	-	3.22±0.19	-	-
Glucosativin	-	-	-	-	1.81±0.14
Indoles					
Glucobrassicin	1.61±0.21	1.21±0.15	0.47±0.16	0.57±0.03	-
4-Hydroxyglucobrassicin	-	-	0.19±0.08	0.20±0.02	-
Total Glucosinolates	29.31±1.6	30.89±1.89	7.94±0.67	1.44±0.08	1.81±0.14

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