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Research Article

Comparative GC-MS Analysis of two *Brassica rapa* L. Varieties for Identification of Volatile Compounds

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Abstract

Plant volatiles are known to play important in herbivore-plant interaction. Extraction and identification of these volatile compounds is very essential for exploring their possible function in plant communication with other organisms. The gas chromatography-mass spectrophotometer (GC-MS) analysis two *Brassica rapa* L. varieties (*B. rapa* var toria and *B. rapa* var yellow sarson) revealed variation in quantity of volatile compounds during different growth stages. Twenty two (octane, Nonane, 2,5-dimethyl-, Undecane, 5-ethyl-, Hexadecane, Heptadecane, Heneicosane, Tetradecane, Tridecane, 3-methyl-, Henecosanol, 9-Octadecene, (E)-, Dodecane, Dotriacontane, Pentadecane, Tetratriacontane, Tetratriacontane, n-Heptadecanol-1, Eicosane, 2,4-dimethyl-, n-Tetracosanol-1, Triacontane, D-Limonene, 2-Hexanone, 3-Hexanol and 2-Hexanol) were identified from hexane extracts of both varieties of *B. rapa*. The concentration of volatile compounds was found higher in pod stage extract than both vegetative and flowering stage extracts. Compounds viz., 3-Hexenone, 3-Hexenol, 2-Hexenol and Dotriacontane were not detected in *B. rapa* var sarson extracts.

Keywords: *Brassica rapa*, gas chromatography-mass spectrophotometer, plant volatiles, toria, yellow sarson

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Introduction

Brassica is an important group of oil seeds in India growing on 6.6 mha areas with 8.3 mt productions [1]. A number of volatile compounds viz., terpenes, glucosinolates, alcohols, aldehydes, esters and ketones are produce from brassica plants [2]. These volatiles benefit the plant by reducing the impact of herbivore or they can attract specific [3]. Many of the compounds in plants are derived from fatty acids, amino acids and carbohydrate [4]. Extraction and identification of volatile compounds is essential for exploring their chemical properties [5]. The study on volatile profile at different growth stage might increase our understanding regarding importance of these volatile compounds in herbivore-plant interaction. Based on this previous knowledge, the comparative volatile profiling analysis of two brassica species (*B. rapa* var toria and *B. rapa* var yellow sarson) during three growth stages was done by gas chromatography-mass spectrophotometer (GC-MS).

Materials and Methods

Host plant and insects: Two variety of *B. rapa* including *B. rapa* var toria and *B. rapa* var yellow sarson used in present investigation were grown in research farm of Indian Agricultural Research Institute (I.A.R.I) New Delhi during 2015-16 (Figure 1).

Extraction and identification of chemical constitutes: The samples (30g) from both variety were collected at different growth stages viz., vegetative stage, flowering stage pod formation stage. The plant extracts of both varieties were prepared based on the protocol used by [6]. Sample of each stage was immersed overnight chilled hexane (250 ml) in freeze followed by filtration through filter paper (whatman no. 1). The filtered elute was further subjected to cleanup by column chromatography. Elute from column was subjected to vacuum evaporator (25-30ºC at 30-35 RPM) to evaporate solvent and final volume obtained (5mL) was stored in -20ºC for further use.

GC-MS analysis: Shimadzu QP 2000 equipped with Rtx-5ms column measuring 30×0.25mm composed of 95% Dimethyl polysiloxane were used for gas chromatography-mass spectrometer (GC-MS) analysis for identification of

volatile compounds. Helium with flow rate 1ml/min was used as carrier gas. One microliter volume of each sample was utilized. The injection temperature was maintained at 230°C. The initial temperature of oven temperature was programmed at 40°C for 4 min, then an increase to 220°C and finally allowed to increase to 270°C with ending rate of 15°C for 1 min. The running time of sample was 45 min. The temperature for ion source was maintained at 200°C. Electron impact ionization (EII) with 70eV was used for GSMS analysis and data was evaluated by TIC (Total ion count) for identification and quantification of compounds. The spectrums of each compound were compared with known stored data base of spectrum in GC-MS library (NIST14) and further data processing and peak areas measurement was done through software (Turbo-Mass-OCPTVS-Demo SPL).

Statistical analysis: Fisher's LSD test was used for separation of treatment mean using https://www.xlstat.com.

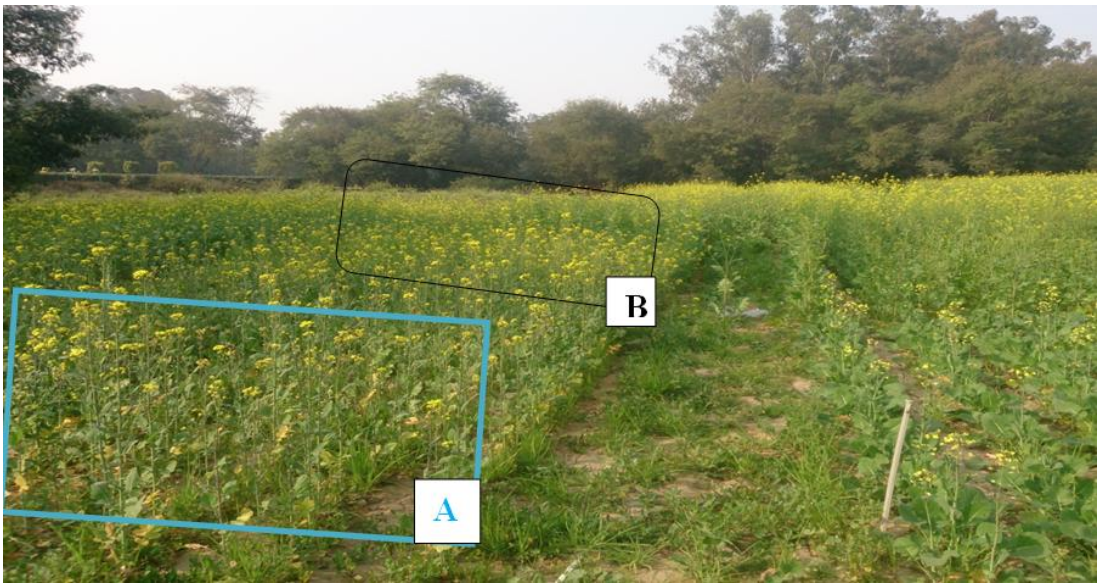

Figure 1 Research farm, Indian Agricultural Research Institute, New Delhi. A=*B. rapa* var toria, B=*B. rapa* var yellow sarson

Results and Discussion

Extraction, Identification and quantification of chemical constituents: The identification and quantification of chemical compounds at different developmental stages of *B. rapa* were done by GC-MS. The results are presented in Table 1 with the per cent area of each compound in all extracts and the GC-MS chromatogram of each growth stage was given separately (Figure 2). A Total of 17 saturated hydrocarbons with carbon number ranging from C_9-C_{54} were identified from *B rapa* var toria with highest 15 compounds identified in pod stage extract. The number of compounds identified from vegetative stage and flowering stage was 14 and 13 respectively (Table 1). Total four compounds viz., 3-Hexenone, 3-Hexenol, 2-Hexenol and Dotriacontane were not detected in *B. rapa* var yellow sarson extracts. Compound Dodecane (1.47%), Heneicosane (1.23%) and Hexadecane (1.18%) were the major constituents in vegetative stage while Octane (5.84), Tetradecane (4.01), Henecosanol (3.75) and Hexadecane (3.41) were the most abundant compounds in flowering stage. The most dominant constituents of pod stage extract were Dodecane (9.92), Octane (6.51), Hexadecane (4.73) and Heptadecane (3.45) (Table 1).

The diversity of compounds was found in different developmental stage of yellow serson extracts. A total 20 saturated hydrocarbon were recorded from three developmental stages of *B. rapa* yellow sarson extracts (Table 1). Similar to *B. rapa* var toria, Octane (1.91), Henecosanol (1.83), Tetradecane (1.82) were found in higher quantity from vegetative stage (Figure 1) extract while Tetradecane (2.87), Hexadecane (2.67) and Octane (2.33) were the most dominant compounds in extract of flowering stage. The most dominant constituents of pod stage extract were Hexadecane (9.22), Octane (7.35), Heptadecane (6.37), Undecane, 5-ethyl (5.82) and Tridecane, 3-methyl (5.64) (Table 1). Three compounds including 2-Hexenone, 3-Hexenol, and 2-hexanol were detected only from flowering stage extract of yellow sarson than other extracts of variety and compounds n-Heptadeacnol-1, Triacontane, and D-limonene were not detected from yellow sarson. D-Limonene and Triacontane from extract of pod stage of *B. rapa* var toria than other extracts.

Table 1 Chemical compounds identified from *B. rapa* species by GC-MS

S. No.	Name of compounds	Concentration of compounds (%)					
		***B. rapa* var toria**			***B rapa* var yellow sarson**		
		Vegetative stage	**Flowering stage**	**Pod stage**	**Vegetative stage**	**Flowering stage**	**Pod stage**
1	Octane	0.97	5.84	6.51	1.91	2.33	7.35
2	Nonane, 2,5-dimethyl-	0.28	3.12	0.21	0.88	0.20	0.27
3	Undecane, 5-ethyl-	0.29	0.54	0.99	0.52	1.61	5.82
4	Hexadecane	1.18	3.41	4.73	1.45	2.67	9.22
5	Heptadecane	0.53	2.28	3.45	0.74	0.70	6.37
6	Heneicosane	1.23	1.22	2.54	0.48	0.64	4.23
7	Tetradecane	0.74	4.01	2.72	1.82	2.87	3.34
8	Tridecane, 3-methyl-	-	0.61	0.85	-	0.64	5.64
9	Henecosanol	1.22	3.75	-	1.83	0.79	-
10	9-Octadecene, (E)-	0.66	0.96	-	0.19	0.30	0.16
11	Dodecane	1.47	3.12	9.92	0.78	1.71	6.96
12	Dotriacontane	-	-	-	-	-	0.15
13	Pentadecane	-	0.80	0.94	0.15	0.76	1.40
14	Tetratriacontane	0.35	-	-	-	-	2.83
15	n-Heptadecanol-1	1.28	2.42	0.19	-	-	-
16	Eicosane, 2,4-dimethyl-	0.95	-	0.18	-	0.81	0.15
17	n-Tetracosanol-1	0.51	-	-	-	0.47	-
18	Triacontane	-	-	0.13	-	-	-
19	D-Limonene	-	-	0.52	-	-	-
20	2-Hexanone	-	-	-	0.17	-	-
21	3-Hexanol	-	-	-	0.16	-	-
22	2-Hexanol	-	-	-	0.16	-	-

- Not detected

The concentration of compounds was found to be similar at vegetative stage (Figure 2) in both varieties but during flowering stage, the concentration of compounds in *B. rapa* var toria was found to be increased than yellow sarson (Table 1 and **Figure 3**). The concentration of compounds remain constant up to pod formation stage in *B. rapa* var toria but it increased more in yellow sarson than toria (Figure 3).

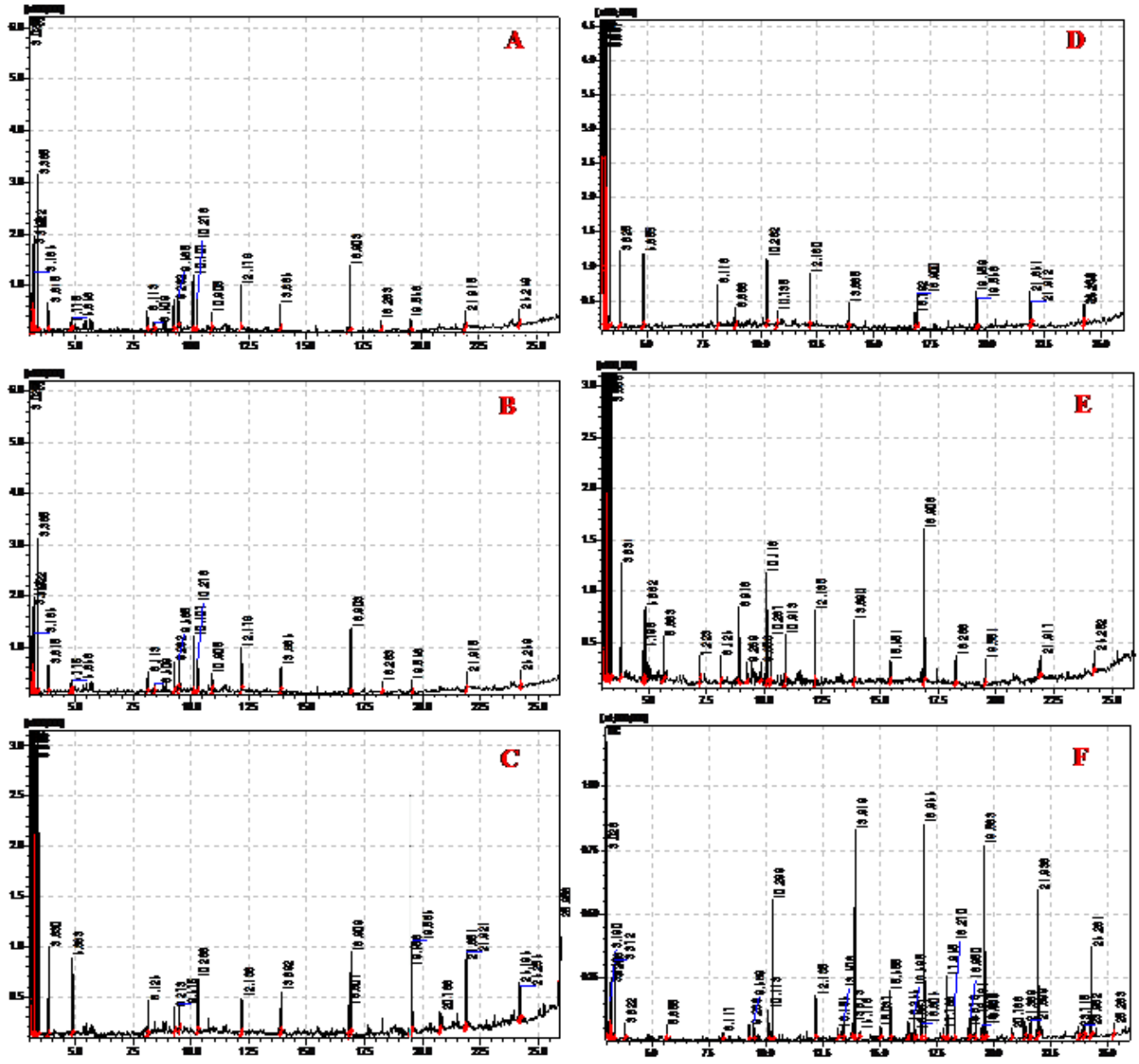

Figure 2 GC-MS chromatogram of *B. rapa* var toria and *B. rapa* var yellow sarson at different developmental stages.

Code for each stage:
A. vegetative stage, B. flowering stage,
C. pod formation stage of *B. rapa* var toria and
D. vegetative stage, E. flowering stage,
F. pod formation stage of *B. rapa* var yellow sarson.

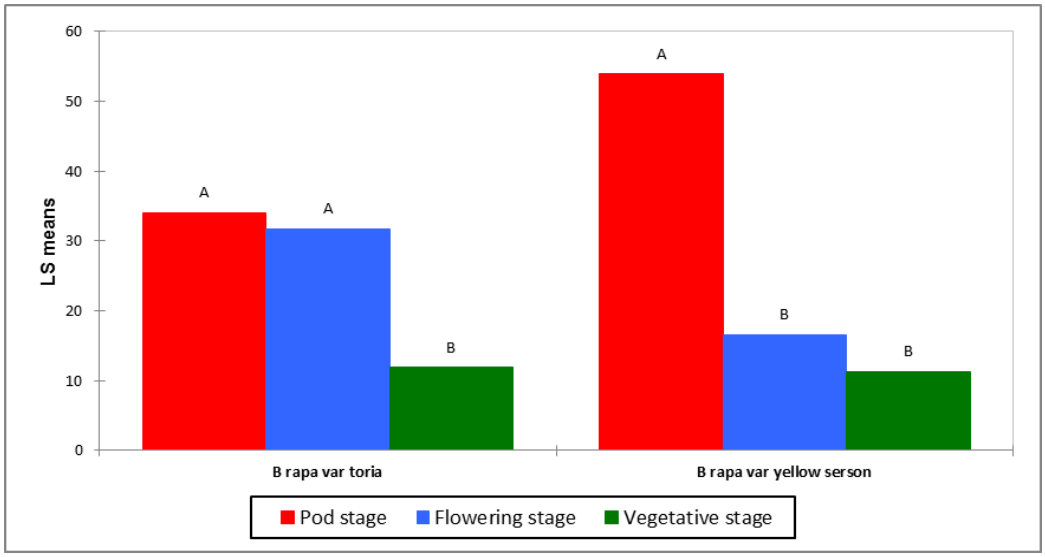

Figure 3 Variation of volatile compounds at different growth stages of *B. rapa* var toria and *B. rapa* var yellow sarson. Mean (n=3) with no letters in common are significantly different (ANOVA followed by Fisher's LSD $P < 0.05$).

The extraction and identification of plant volatiles play significant role in better understanding of insect plant interaction owing to the diversity and variation of volatiles in different plant part [6]. These volatile compounds work as a chemical cues used by plants for their interaction with other surrounding organisms. They play significant role in herbivore defence against a wide spectrum of enemies [7] and some of them guide the towards host plants. An experiment conducted by [8] revealed that (±)-2-hexanol extracted from the leaves of *Pterocarpus indicus* Willd elicited a strong response from the female *Aleurodicus disperses* Russell antennae. Moreover, Y-tube olfactometer study shows that the female *A. dispersus* was attracted in response to different concentrations of (±)-2-hexanol but (*Z*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol act as a repellent against *Dendroctonus valens* LeConte [9]. Previous study conducted by [10] have found phagostimulatory nature of compounds 1-hexanol, (*Z*)-3-hexen-l-ol and (*Z*)-2-hexen-l-ol from essential oil (*Solanum campylacanthum* Hochst) against the larvae of *Epilachna fulvosignata* Reiche. In a series of experiments [11] found generalization of a conditioned response from one functional odour to another molecule. Compounds 2-hexanone or 1-decanol tested with a number of alcohols and ketones for conditioning of moths that revealed the generalization of the conditioned response decreased as a function of the chain length and functional group. Volatile compounds 9,12,15 octadecatrienoic acid and 9-octadecenal from rice cultivars might have played positive role in the attraction of *T. chilonis* Ishii while Hexadecane, Heptadecane, Petadecane and Hexadeconic acid might be responsible for the attraction of *T. japonicum* Ashmead [12]. The dual choice flight orientation study indicated that saturated hydrocarbons (Hexadecane, Hexacosane, Nonacosane, Pentacosane, Heneicosane, Tricosane, Triacontane, Heptacosane, Tetracosane and Docosane) were found to be attractive against female, *Cotesia plutellae* Kurdjumov [13].

Conclusion

Herbivore insects utilize the volatile compounds released from the plant to locate suitable host plant and these volatile compounds also help the plant against herbivore insects. Thus extraction and identification of these active compounds not only improve understanding of insect-plant but also helps us to find the best possible combinations (plant extracts with synthetic compounds) of the most effective volatile compounds for management of insect pests and as well as to manipulate the behaviour of their natural enemies.

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