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# Morphological, microscopic and chemical comparison between *Nigella sativa* L. cv (black cumin) and *Nigella damascena* L. cv

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**Abstract**

The seeds of *Nigella sativa* L. have been shown in both *in-vitro* and *in-vivo* to possess interesting pharmacological properties. These properties have been attributed to the volatile fraction, the principal component of which is thymoquinone. If clinical studies of *N. sativa* are to be envisaged it will be necessary to develop a means to ensure the homogeneity of different sources and to avoid confusion, notably with *N. damascena* the seeds of which closely resemble those of *N. sativa*. Gas chromatography coupled to mass spectrometry was used to analyse the volatile and fixed oil composition of different sources of *N. sativa* seeds. There was a substantial variation in the volatile fraction between the different sources, whereas the fixed oil composition remained relatively constant. In addition, the GC chromatograms of volatile fractions of *N. sativa* and *N. damascena* were remarkably different; the presence of the toxic compound, damascene was confirmed in *N. damascena* and thymoquinone was shown to be absent from this species of *Nigella*. A morphological and microscopic examination of *N. sativa* and *N. damascena* seeds showed for the first time that physical differences barely discernible on to the naked eye are readily identifiable using a 20-fold magnification.

***Key words:*** *Nigella sativa* L., compositional variation, microscopy, GC-MS.

## Introduction

The genus *Nigella* L. includes some 20 species distributed from the Mediterranean regions to west Asia [1]. The term "*Nigella*" comes from the Latin *niger*, a reference to the intense black colour of their seeds. *N. sativa* L., commonly known as "black seed" or "black cumin", is used in folk medicine as a natural remedy for a number of diseases and conditions such as asthma, hypertension, diabetes, inflammation, bronchitis, headache, eczema, fever, dizziness and gastrointestinal disturbances. Its many medicinal uses have been the subject of various review articles [2-6].

The main constituents of *N. sativa* are alkaloids, and fixed and volatile oils. El-Alfy *et al.*[7] found that *N. sativa* contains 0.4% volatile oil calculated on the basis of the dry weight of the seeds. The most abundant components in the volatile fraction of *N. sativa* seeds are the pharmacologically active quinone, thymoquinone (TQ), and *p*-cymene. It has also been shown that compounds isolated from *N. sativa* (including thymoquinone, carvacrol, t-anethole and 4-terpineol) have appreciable free radical scavenging properties [8]. Thymoquinone can prevent oxidative injury in hepatocytes induced by carbon tetrachloride or tert-butyl hydroperoxide in various *in vitro* [9] and *in vivo* [10] hepatotoxicity models.

Although the chemotherapeutic potential of TQ in the clinic has not been tested, numerous studies have shown the anti-cancer effects of *N. sativa* in animal models [11, 12]. Gali-Muhtasib *et al.* [13] showed that thymoquinone was effective in inhibiting the growth of HCT-116 human colon cancer cells, and the combination of TQ with clinically used anti-cancer drugs lead to improvements in their therapeutic index and prevented non-tumour tissues from sustaining chemotherapy-induced damage. Quite recently, researchers at the Kimmel Cancer at Jefferson Center for Pancreatic, Biliary and Related Cancers have found that thymoquinone blocked pancreatic cancer cell growth and killed the cells by enhancing the process of programmed cell death (unpublished results). While the studies are in the early stages, the findings suggest that thymoquinone could eventually have some use as a preventative strategy in patients who have gone through surgery and chemotherapy, or in individuals who are at a high risk of developing cancer.

*N. sativa* has been frequently reported as being effective in lowering blood sugar [14-19]. Hawsawi *et al.*[15] showed that both *N. sativa* and thymoquinone produced significant hypoglycaemic effects in diabetic and normal rats, and Al-hader *et al.*[20]obtained similar results by administrating the volatile oil of *N. sativa* seeds (50 mg $kg^{-1}$) to fasting normal and alloxan-diabetic rabbits. Le *et al.* [17] demonstrated that petroleum ether extracts of *N. sativa* exerts lipid-lowering and insulin-sensitising actions in rats. Studies in humans are rare [21] but anecdotal evidence suggests that *N. sativa* has been traditionally used to treat diabetes in indigenous communities [19, 22]. The exact mechanism of action has not been elucidated but El-Dakhakhny *et al.*[23] reported that the hypoglycaemic effect of *N. sativa* oil is mediated by extra-pancreatic actions, whereas other *in-vitro* studies indicate that

the anti-diabetic properties of *N. sativa* seeds may be at least partly mediated by stimulated insulin release [24].

The seeds of *N. sativa* strongly resemble those of an another species of the *Nigella* genus, *N. damascena* which is also used for ethno-therapeutic purposes, though the seeds of *N. damascena* do not contain thymoquinone. *N. damascena* differs substantially in its composition from *N. sativa* by its sesquiterpenoid contents [25-27], which consist solely of 15 carbon atom units, and by the presence of anthranilic acid derivatives. Sesquiterpenes, such asgermacrene A, β-elemene and five selinenes, were detected in abundant amounts [28]. Two of these anthranilic acid derivatives are the alkaloids, damascenine and damascinine which are considered to be toxic [29]. Furthermore, the seeds of *N. sativa* are characterized by a very low degree of toxicity[2,30].

Given the medicinal properties of *N. sativa*, clinical studies are being considered particularly in relation to its anti-diabetic properties. The originality of this study resides in the establishment of a relatively simple microscopic method to distinguish between *N. sativa* and *N. damascena* seeds given their similarity to the naked eye, and the possibility therefore of confusion or accidental adulteration. The macroscopic and microscopic distinction between the two species was confirmed by gas chromatography-mass spectrometry. The second objective was to investigate the variability in the concentration of the active principle according to where the seeds were sourced, as clearly if this seed is to be of use therapeutically, it will be necessary to develop a means to standardise the extract.

## Materials and Methods

***Samples and reagents:*** Hexane, dichloromethane (DCM) and methanol HPLC grade were purchased from Prolabo, France; KOH, RPE grade from Carlo Erba, Italy. Thymoquinone, carvacrol, trans-anethole, α-pinene and *p*-cymene were obtained from Sigma Aldrich. Olive oils, 2005 harvest were provided by the Olive Oil Cooperative of Caunes-Minervois, France and a commercial mixture of different plant oils and containing 50% olive oil, 30% sunflower oil, 2% raisin oil and 15% oil seed rape were used as standards for analysing the lipid profile.

*N. sativa* seeds used in the study were obtained from different suppliers. Whole *Nigella* seeds obtained from a wholesaler were of Indian origin (Sample I). Whole *N. sativa* seeds (Sample II) and *N. sativa* powdered seeds (Sample III) were purchased in a local pharmacy and were sourced in Syria. Sample IV was obtained from Morocco and Sample V from Tunisia. Other *N. sativa* seeds were purchased from different herbalists in Montpellier, France. Samples VI and VII correspond to two lots from the same supplier in France. Samples VIII and IX were obtained from two other suppliers in France. It was not possible to determine the exact origin of samples VI-IX. *N. damascena* seeds were supplied by Voltz Seeds, the Netherlands.

***GC analysis:*** For GC analysis of the DCM extractable fraction, 1 g of ground Nigella seeds was mixed with 5 ml of dichloromethane in an amber container. The container was sealed and stored for six days and agitated with periodic homogenisation. The samples were filtered through paper and the filtrate was introduced directly into the gas chromatograph.

For GC analysis of the fatty acids, methyl esters of the fatty acids were prepared. After grinding, 1 g of the *Nigella* seeds was mixed with 2 ml hexane followed by the addition of 0.2 ml of 2M methanolic KOH. The mixture was vortex mixed for two min at room temperature.

A Trace GC Thermo Quest Gas chromatograph coupled to a Trace MS plus mass selective detector was used for GC-MS analysis. Separations were carried out on a SGE BPX 5 capillary column (30 m x 0.25 mm I.D., 0.25 μm). The chromatographic conditions presented in Table 1 were used. Identification was by comparison with standards or, if standards were unavailable, with the library of mass spectra. For the fatty acid composition, the retention indices of the fatty acid methyl esters were determined relative to the retention times of fatty methyl esters from commercial edible oils and by mass spectrometry.

***Repeatability of the volatile fraction analysis:*** In order to test the repeatability of the method for the volatile fraction, five repetitions of the same sample were analysed. Approximately 4 g of crushed seeds were placed in 20 ml dichloromethane. After 6 days' agitation in sealed amber containers, the solvent was evaporated to approximately 1 ml and analysed by GC-MS.

***Microscopy:*** The seeds of both *Nigella* species were first examined using a Paralux binocular magnifier fitted with a WF10X eyepiece and two objectives (2X and 4X). Closer examination of the different tissue parts was carried out using a LEICA DME light microscope with a Periplan eyepiece 10V/18 and LEICA ACHRO objectives ofX0.1, 10X/0.22, and 40X/0.65. Photographs were taken with a CANON Power Shot 82 attached to the LEICA microscope.

## Results and Discussion

***Morphology:*** Fig. 1 shows that the seeds of *N. sativa* and *N. damascena* are very similar to the naked eye, and thus a systematic study was carried out both macroscopically and microscopically in order to determine which properties could be used to distinguish between the two species.

The macroscopic characteristics are presented in Table 2 and may also be observed in Fig. 2. As may be seen from Fig. 2a, the seeds of *N. sativa* are pear-shaped with one flat side and one convex side, whereas the seeds of *N. damascena* are oval and both sides are curved (one more so than the other); they are also

**Table 1.** GC analytical conditions.

| | |
|---|---|
| Initial temperature | 50 |
| Initial time | 5.00 |
| Rate | 5.0 |
| Final temperature | 270 |
| Hold time | 15.00 |
| Equilibration Time | 0.50 |


***Figure 1.*** *N. damascena* (a) and *N. sativa* seeds (b).

**Table 2.** Macroscopic and microscopic characteristics of *N. sativa* and *N. damascena* seeds.

| | *N. sativa* | *N. damascena* |
|---|---|---|
| Shape | Pear-shaped with slightly curved tapered ends. One side is flat and the other is convex. The surface is slightly and regularly embossed | Ovoid shape with one side very slightly more curved than the other. Embossed surface with veins only on the less curved side (Fig. 4b) |
| Colour | Black with hints of light grey | Anthracite black |
| Size (mean of 25 samples) | Length 4.1 cm, Width 2.0 cm | Length 3.0 mm, Width 2.3 cm |
| | Transversal cross-section often hexagonal; longitudinal cross-section is pear-shaped | Rounded transversal cross-section; longitudinal cross-section is oblong |
| Flavour and evolution of taste | Metallic taste when the seed comes into contact with dental enamel. After crushing, taste of lead pencil, followed by sharp, aromatic peppery taste, becoming irritant at the base of the throat and leaving a very persistent bitterness on the palate. | Metallic taste when the seed comes into contact with teeth. After crushing with the teeth, persistent taste of dried apple, strong aromatic taste which fills the mouth and persists on the palate. |
| Crushing | Easy with dissociation of tissues | Difficult with tissues remaining intact |
| Microscopic observation | -Brick red external tegument consisting of polygonal cells (penta- to heptagonal) which are very slightly embossed<br>-Grey-coloured albumen consisting of thin-walled cells. Several oil droplets<br>- Tissue surrounding the albumen is orangey-brown consisting of a single layer of polygonal cells (square) which are often aligned | - Brick red external tegument consisting of cells that vary from rounded to polygonal which are both embossed and contain ridges. Some of cells of the tegument are longer and grouped into a prominent network.<br>- Greyish albumen consisting of thin-walled cells. Several oil droplets.<br>- Tissue surrounding the albumen is orange-brown consisting of a single layer of thick-walled polygonal cells. |

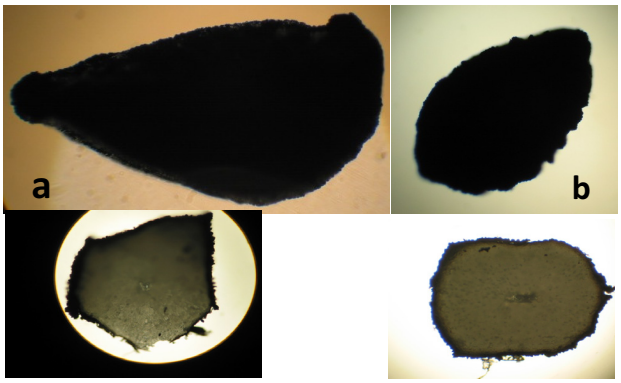


***Figure 2.*** *N. sativa* (a) and *N. damascena* seeds (b) Magnification 20x. Details in Table 2a.

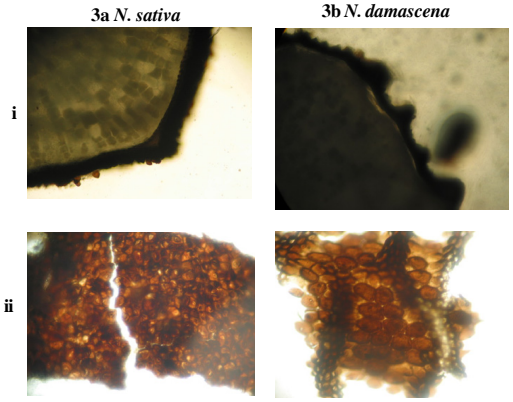


***Figure 3.*** Cross-sections of the external tegument of *N. sativa* (a) and *N. damascena* (b) Magnification 400X. Details in Table 2.

have a slightly more homogeneous colour than *N. sativa* and are smaller in length (3.0 cm as opposed to 4.21 cm for *N. sativa*). Both seeds are highly aromatic, but those of *N. sativa* differ from those of *N. damascena* in that they are more peppery, irritant and bitter. Microscopic characteristics (cross-sections) are presented in Table 2 and Fig. 3. It may be observed that in *N. sativa* the external tegument consists of polygonal cells (penta- to heptagonal) which are very slightly embossed (Fig. 3a)i, whereas in *N. damascena* the external tegument consists of cells that vary from rounded to polygonal and are both embossed and contain ridges. Some of cells of the tegument are longer and are grouped in into a prominent network which may be clearly seen in Fig. 3(b)ii.

***Composition:***

*Repeatability of the volatile fraction analysis:* Five replicates of the sample *N. sativa* were analysed by GC-MS and as may be seen in Table 3 the repeatability (expressed in terms of the RSD%) is, with the exception of pinene, less than 5% for all the compounds of interest. Therefore, all further analyses were carried out singly or in duplicate. The principal volatile constituents were identical in the majority of the *N. sativa* samples analysed, however, there was considerable variation in their quantities as may be seen in Fig. 4 (Samples VI and VII).

Thymoquinone and *p*-cymene were the most abundant compounds in the DCM extracts of all seed samples (Fig. 5). The concentration of thymoquinone varied from 30.6% (Sample V) to 82.4 % (Sample VIII). These results agree with other reports in the literature [31, 32], and Al-Saleh *et al.* [33] reported that the concentration of thymoquinone varied markedly depending on the country of origin. The second most abundant constituent was *p*-cymene which was present in all extracts in concentrations ranging from 13 to 34% of the cumulative volatile compounds detected. Some volatile compounds were only detected in particular samples, such as α- terpinene (Sample VI) p-allyl anisole (Sample VII) and carvon (Sample III) whereas α-pinene, β-pinene,

**Table 3.** Repeatability of the volatile fraction analysis.

| Compound | Sample | TR (mins) | Area | % in the sample | $\bar{x}$ | $\sigma_{n-1}$ | C.V. % |
|---|---|---|---|---|---|---|---|
| Thymene | 1 | 7.93 | 22030075 | 10.9 | | | |
| | 2 | 8.01 | 89497954 | 10.3 | | | |
| | 3 | 8.01 | 10554106 | 10.0 | 10.24 | 0.3975 | 3.9 |
| | 4 | 8.06 | 20543131 | 10.1 | | | |
| | 5 | 8.08 | 75633966 | 9.9 | | | |
| Pinene | 1 | 8.17 | 7295225 | 3.5 | | | |
| | 2 | 8.23 | 22899175 | 2.5 | | | |
| | 3 | 8.23 | 2530840 | 1.9 | 2.64 | 0.6229 | 23.6 |
| | 4 | 8.28 | 6275675 | 3.0 | | | |
| | 5 | 8.29 | 18943209 | 2.3 | | | |
| Cymene | 1 | 11.62 | 72040922 | 35.8 | | | |
| | 2 | 11.68 | 312911081 | 36.2 | | | |
| | 3 | 11.72 | 40086090 | 39.6 | 37.38 | 1.7035 | 4.5 |
| | 4 | 11.75 | 72503182 | 36.5 | | | |
| | 5 | 11.76 | 293808553 | 38.8 | | | |
| Unidentified peak | 1 | 14.72 | 12080394 | 5.9 | | | |
| | 2 | 14.74 | 42383491 | 4.8 | | | |
| | 3 | 14.81 | 4887445 | 3.9 | 4.98 | 0.7328 | 14.7 |
| | 4 | 14.81 | 10737999 | 5.0 | | | |
| | 5 | 14.81 | 40286923 | 5.3 | | | |
| Thymoquinone | 1 | 19.13 | 88332388 | 43.8 | 44.56 | 0.9736 | 2.2 |

**Table 4.** Overview of the volatile composition of the 9 samples.

| | Sample | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX |
| 4- Terpineol | 0.7 | 0.6 | 5.6 | 0.9 | 0.8 | 0.9 | | | |
| α- Pinene | 7.4 | 6.8 | 6.7 | 12.2 | 7.4 | 4.9 | 2.7 | | |
| α- Terpinene | | | | | | 0.5 | | | |
| Anisole-p-Allyl | | | | | | | 0.5 | | |
| β-Pinene | 2.1 | 1.9 | 3.0 | 3.5 | 2.2 | 1.6 | 1.7 | | |
| Bornylacetat | | | | 0.3 | 0.4 | 0.2 | 0.2 | | |
| Camphene | 1.1 | | 3.2 | 1.6 | 0.7 | 0.9 | 0.6 | | |
| Camphor | 1.1 | 1.2 | | | 0.7 | 1.5 | 1.1 | | |
| Carvacrol | | | 2.6 | 4.7 | 5.1 | 4.0 | 1.4 | | |
| Carvon | | | 2.9 | | | | | | |
| Limonene | 2.8 | 1.6 | 3.4 | 2.7 | | 2.5 | 2.4 | | |
| Longifolene | 1.7 | 1.8 | 3.2 | 0.9 | 1.7 | 4.8 | 1.9 | | |
| Longipinene | | 0.8 | | 0.2 | 0.4 | 1.1 | 0.4 | | |
| p-Cymene | 26.0 | 18.8 | 16.3 | 30.02 | 33.8 | 19.2 | 13.6 | 14.5 | 20.3 |
| p-Cymen-8-ol | | | | 0.3 | 0.1 | 0.2 | 0.1 | | |
| Sabinene | 0.2 | 1.7 | 2.4 | 3.0 | 1.6 | 1.1 | 0.7 | | |
| Thujone | | | | | 0.3 | 0.5 | 0.6 | | |
| Thymoquinone | 49.9 | 55.7 | 39.1 | 32.7 | 30.6 | 47.9 | 45.5 | 82.4 | 73.6 |
| t-Anethol | | 2.8 | 4.9 | | 0.2 | | 19.4 | | |
| Unknown 1 | 1.1 | 0.8 | | 0.9 | 0.8 | 0.8 | 0.7 | | |
| Unknown 2 | 4.5 | 4.2 | 4.7 | 5.6 | 4.9 | 5.0 | 3.7 | 3.1 | 6.1 |
| γ-Terpinene | 1.5 | 0.4 | 2.1 | 0.5 | 8.3 | 2.5 | 2.2 | | |

Values are relative percentages of the total content extracted by dicholoromethane. Peaks other than thymoquinone, carvacrol, trans-anethole, α-pinene and para-cymene were identified by reference to the mass spectral library.

limonene, longifolene and sabinene were found in Samples I-VII.

However, from examination of Table 4, which presents a summary of the compounds found in the DCM extracts, it may be observed that the samples may be grouped according to the distribution of the different volatile compounds in the DCM extracts. Samples I, II, VI and VII contain similar (46-55% of cumulative volatile compounds detected) amounts of thymoquinone and *p*-cymene (13-25%).

Samples IV and V contain approximately 30% of both thymoquinone and *p*-cymene, whereas Samples VIII and IX, on the other hand, contain between 70 and 80% thymoquinone and 15-20% *p*-cymene. This suggests that in general, the greater the amount of thymoquinone present, the lower the concentration of *p*-cymene. This compound has been shown to reduce blood pressure in rats [34] and has also been reported to posess a local anaesthetic effect [35] and mild antibacterial activity [36]. The fact that the pharmacological properties of *p*-cymene are not identical to those of thymoquinone, notably in terms of anti-diabetic effects, could result a variation in the pharmacological actions among different batches of *N. sativa* seeds according to the relative proportions of thymoquinone and *p*-cymeme. Sample III is a powdered version of Sample II, and it may be seen from Table 4 that the relative proportions of the principal constituents are lower in this sample, 39.1% *vs* 55.7% and 16.3% *vs* 18.8% for thymoquinone and *p*-cymene, respectively. On the other hand, the relative proportions of constituents present in lesser quantities (<5%) are greater in the powdered form by comparison with intact seeds. A possible explanation is that the grinding process releases lesser compounds from cell vacuoles, a theory which may merit further investigation.

The DCM extracts of *N. damascena* seeds shows the presence of damascenine (retention time 27.4 min., Fig. 6) identified by its mass spectrum and which is absent from *N. sativa*. Furthermore, it may be observed that thymoquinone, the principal constituent in the volatile extract of *N. sativa* is absent from *N. damascena* which demonstrates that the morphological similarity of the seeds of both species is in no way reflected in the composition of the DCM extracted fraction

*Fatty acid composition*: All samples contain the same fatty acids, which were detected as fatty acid methyl esters (after derivatisation) as described in the experimental section. The principal acids were the saturated fatty acids, palmitic (16:0), stearic (18:0) and arachidic acid (20:0) and the unsaturated fatty acids linoleic (18:2) and oleic acids (18:1). Although the specific amount of each fatty acid varied between samples, linoleic acid which accounted for about 50 % was the predominant fatty acid, followed by oleic and palmitic acids. Interestingly, there appeared to be an inverse relationship between the relative percentages of linoleic acid, an ω-6 unsaturated fatty acid and oleic acid an w-9 unsaturated fatty acid (Fig. 7). The high content of the unsaturated fatty acids is nutritionally desirable. Aurand *et al*.[36] reported that the nutritional value of linoleic acid is due to its metabolism at tissue levels which produce the hormone-like prostaglandins, whose pharmacological properties include lowering blood pressure and constriction of smooth muscle. These results confirm those of Ramadan and Mörsel [37] who fractionated the main lipid components of the seeds. Ceikh-Rouhou *et al*. [38] also reported that the major unsaturated fatty acids in crude oil extracts of *N. sativa* were linoleic acid followed by oleic acid while palmitic acid was the main saturated fatty acid. There was very little variation in the fatty acid composition, suggesting that there is no

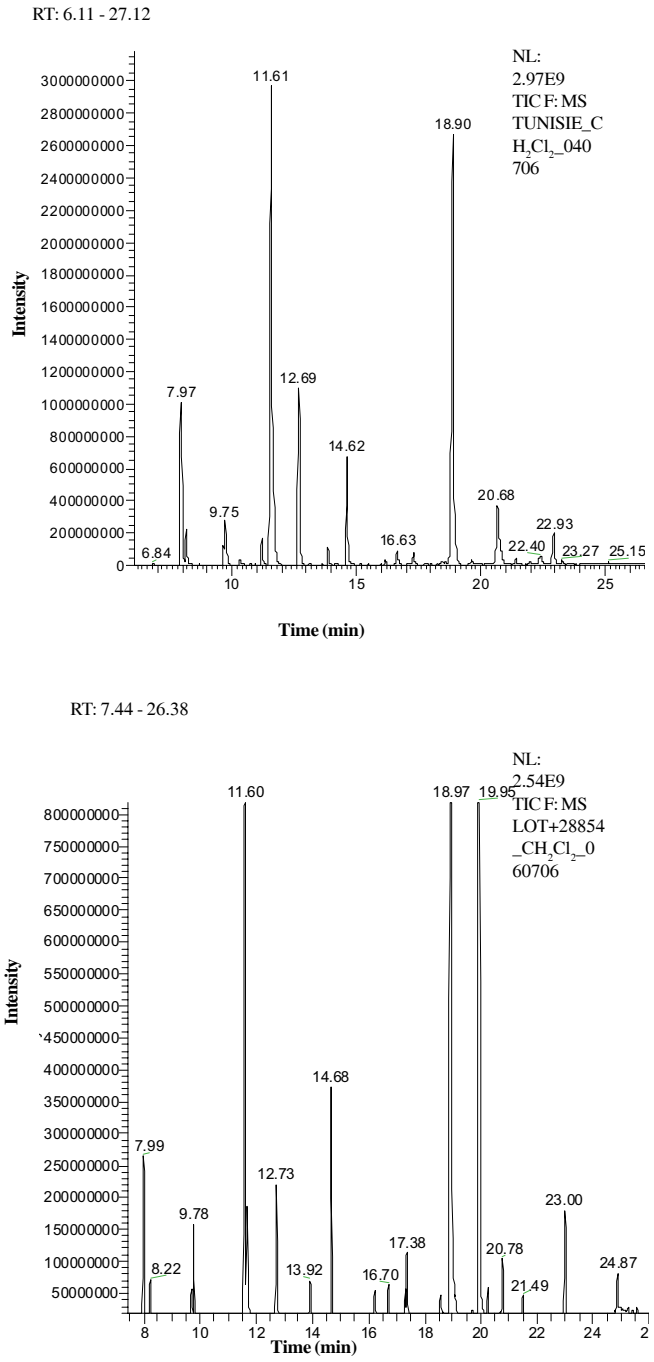


***Figure 4.*** Chromatograms of the volatile composition of *N. sativa* Sample V (a) and Sample VII (b).Peak identification by retention time (min.): 11.7: p-cymene; 14.7: p-mentha-4(8)-en-9-ol; 19: p-mentha-3,6-diene-2,5 dione (Thymoquinone); 20.8: carvacrol; 23:

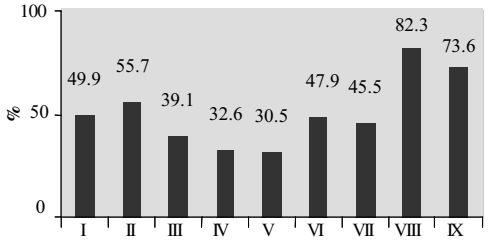


***Figure 5.*** Thymoquinone as a percentage of total volatile composition in Samples I -IX.

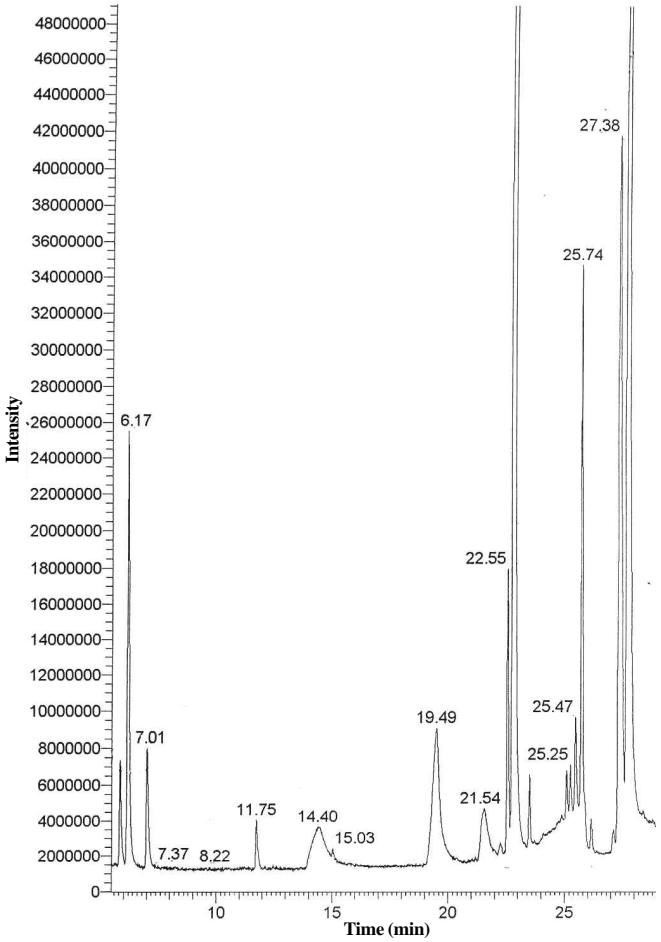


***Figure 6.*** Chromatogram of the volatile content N. damascena seed extract. The peak at 27.38 mins is damascenine.

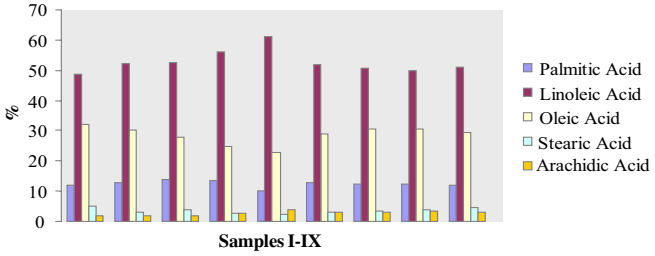


***Figure 7.*** Distribution of fatty acid composition of *N. sativa.*

relationship between this fraction and the volatile components extracted by dichloromethane.

Gas chromatograms of the lipid fractions of the two samples presented in Fig. 4 are shown in Figs. 8a-b. As may be seen, contrary to the volatile constituents which differed significantly between both samples, their fatty acid profiles are almost identical. Other identified fatty acids were detected in trace amounts, and their presence may possibly be accounted for by myristic, myristoleic, palmitoleic, margaric, margaroleic, stearic, linolenic, arachidic, eicosenoic, behenic and lignoceric acids as previously reported [38].

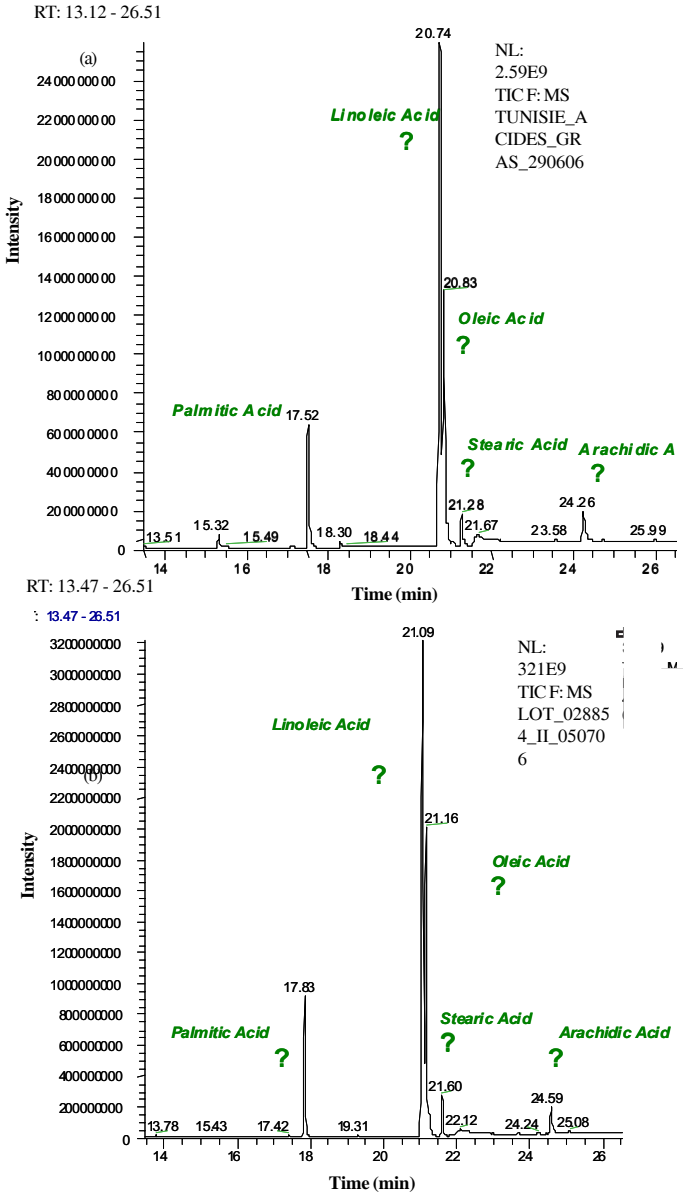


***Figure 8.*** Gas chromatograms of fixed oil composition, sample V(a) and sample VII (b).

## Conclusions

Thymoquinone and *p*-cymene represented a high percentage of the volatile oil fraction in the seeds of all *N. sativa* samples. Other volatile constituents detected were α-pinene and *t*-anethole. It was observed that in comparing *N. sativa* seeds of different origins, there was a significant difference in the concentration and profile of the volatile fraction in the various samples, which may be attributable to the age of the sample, though this hypothesis remains to be confirmed. These observations have considerable implications for clinical studies on the pharmacological effects of the *N. sativa* extracts: unless the extracts are standardised in terms of the concentration of the active principle(s), it will not be possible draw coherent conclusions from any such study. This study has shown that *N. sativa* and *N. damascena* may readily be distinguished by the profile of their volatile fraction in GC-MS, analysis - the alkaloid damascenine was shown to be present in *N. damascena*, but absent from *N. sativa*. Moreover, the *N. damascena* volatile extract did not contain thymoquinone one of the active principles of *N. sativa*. However, GC-MS is not a tool that could be considered feasible for routine control of *N. sativa* seeds in a clinical context. Visual analysis does not easily enable *N. sativa* and *N. damascena* seeds to be distinguished, whereas simple microscopic analysis with a magnification power 20X does permit this distinction. This approach is advisable before undertaking any studies into the clinical effectiveness of this plant in treating diabetes.

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