Comparative evaluation of different *Allium* accessions for allicin and other allyl thiosulphinates

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ABSTRACT

Allicin and other allyl thiosulphinates possess broad antimicrobial and health-promoting properties, which makes them natural and safe substitutes for synthetic preservatives. These thiosulphinate compounds were investigated in 33 *Allium* accessions representing 14 species, and these species differed significantly in their content of allicin, allyl methyl thiosulphinates (AMThs), and allyl trans-1-propenyl thiosulphinates (ATPThs) as ascertained using liquid chromatography tandem mass spectrometry. Total thiosulphinates were the highest in *Allium sativum*, *Allium guttatum*, *Allium tuberosum*, and Zimmu (an interspecific hybrid of *Allium cepa* L. and *Allium sativum* L.). Allicin and other allyl thiosulphinates content was higher in flat and non-waxy leaves than in fistular and waxy leaves. Total thiosulphinates content was also significantly higher in cultivated and semi-domesticated species. Cluster analysis revealed that both foliage type and thiosulphinate content had played a major role in the clustering. These findings are useful in finding alternative sources of allicin and other thiosulphinates, and some of the accessions may serve as sources of natural preservatives for application in other research-related or commercial activities.

1. Introduction

Since ancient times, several species of the genus *Allium* have been known for their medicinal properties, which are due to the presence of organo-sulfur compounds, mainly cysteine sulfoxide (Block et al., 1992). These organo-sulfur compounds, through enzymatic reactions, form thiosulphinates, which are the major flavouring compounds in *Allium* (Lawson and Gardner, 2005; Lawson et al., 1991a, 1991b); the type of thiosulphinates and their ratio vary with the species. Thiosulphinate compounds have many uses: their antimicrobial activities cover both Gram-positive and Gram-negative bacteria and extend to fungi, viruses, and protozoa as well (Bakri and Douglas, 2005; Leontiev et al., 2018). The compounds are also known to possess antioxidant, anti-cancer, and anti-tumour properties, due to several different mechanisms, and therefore play an important role in health and in disease prevention, given the range of their biological activities (Gruhlkea et al., 2019; Santas et al., 2008; Wang et al., 2013). The antimicrobial

and health-promoting properties of these compounds may also make them a natural and harmless alternative to synthetic preservatives to extend the shelf life of food products. Most synthetic food preservatives have multiple side effects on human health because of residual toxicity, and consumers increasingly demand safer alternatives in the form of natural preservatives to replace synthetic chemicals (Zuzarte et al., 2013). The antimicrobial properties of plant products are well documented and have been used for food preservation and in medicine for centuries (Tiwari et al., 2009). With the increasing interest in natural and biologically active compounds as a substitute for synthetic chemicals in the food industry, thiosulphinates offer a particularly attractive option (Benkeblia and Lanzotti, 2007).

Thiosulphinate concentration has been examined in many cultivated species of *Allium* such as garlic (*Allium sativum*; Khar, et al., 2011), ramp (*Allium tricoccum*; Calvey et al., 1997), and onion and bunching onion (*Allium cepa*; Block et al., 1992). Thiosulphinate content of garlic is known to vary (Khar et al., 2011; Sterling and Eagling,

Table 1

LC-MS/MS parameters for the analysis of AMThs, allicin, and ATPThs.

Compound Name	DP, EP (V)	Q > Q1 (CE, CXP (V))	Q > Q2 (CE, CXP (V))	Q > Q3 (CE, CXP (V))
AMThs	30,4	137 > 73 (15, 11)	137 > 95 (10, 11.7)	
Allicin	55,6	163 > 73 (17, 3)	163 > 87 (10, 6)	163 > 121 (10, 13)
ATPThs	50,7	163 > 87 (11, 6)	163 > 121 (11, 13)	163 > 73 (17, 3)

2001), but similar information on some other *Allium* species is scarce and, in many, is missing altogether (*A. altaicum, A. fasciculatum, A. guttatum, A. ledebouranum,* and Zimmu). It is against this background that the present study sought to examine, as one of its objectives, the concentration of three major thiosulphinates, namely allicin, allyl methyl thiosulphinates (AMThs), and allyl trans-1-propenyl thiosulphinates (ATPThs), in leaves of different *Allium* species.

Conservation and maintenance of wild *Allium* germplasm, because of its potential source of many novel and useful traits, is essential for *Allium* breeding programmes (Khrustaleva and Kik, 2000; Kik, 2002). Since wild *Allium* species are important genetic resources for the improvement of cultivated *Allium*, evaluation and characterization of wild germplasm is particularly important—which is why the present study was based on 33 *Allium* germplasm accessions representing 14 species of *Allium*.

2. Materials and methods

2.1. Allium germplasm

The collection and conservation of *Allium* germplasm by the Indian Council of Agricultural Research (ICAR) began in 2010. The collections were made through the ICAR's National Bureau of Plant Genetic Resources (NBPGR) and from a nationwide survey. Many accessions of *Allium* (fifty in number) have been collected and preserved in the field by the ICAR's Directorate of Onion and Garlic Research (ICAR-DOGR) at Rajgurunagar, near Pune, India. These lines are grown and maintained on the DOGR's experimental farm (32 ˚N, 73.51 ˚E; 553.8 m above the mean sea level). A randomized complete block design with three replications was used for field planting of the *Allium* germplasm. Plants of each genotype were planted in a separate block, 40 plants making each replication, at a spacing of 0.5 m × 0.5 m. All the recommended farm management practices were followed, and were the same for all the blocks. The experiment comprised 33 *Allium* accessions representing 14 species of *Allium*. The accessions were divided on the basis of leaf morphology into two groups, one with fistular and waxy leaves and the other with flat and non-waxy leaves, and also, on the basis of the degree of domestication, into four groups, namely wild types, landraces, semi-domesticated, and cultivated (Table S1, supplementary data).

2.2. Reagents and materials

Certified reference standard of alliin (S-alliin-L-cysteine sulphoxide) having purity > 90% was procured from Sigma-Aldrich, Steinheim, Germany. MS grade solvents, acetonitrile and methanol, were purchased from J.T. Baker (Center Valley, USA). In house produced HPLC grade water (Sartorius, Göttingen, Germany) was used for mobile phase preparation. Stock solution of alliin (10 mg/mL) was prepared in water and stored at −20 ˚C.

Standard stock solution of allicin was prepared from alliin stock solution (10 mg/mL) by mixing 1 mL standard alliin solution with 0.5 mL of crude alliinase (aqueous garlic bulb extract devoid of allyl thiosulphinates). The mixture was kept for 15 min at room temperature over a shaker followed by addition of 0.5 mL of acetonitrile (Khar et al., 2011). The mixture was centrifuged for 5 min and the supernatant was stored at -80 ˚C until used. The allicin concentration was calculated as per the following formula:

Concentration of Allicin (Vc) (mg/mL) = (C × 162.28 × Vi × 1000)/ (177.21 × Vf × 2) × 1000

Where, C is the concentration of alliin solution in g/mL; 162.28 and 177.21 are the molecular weights of allicin and alliin, respectively; Vi is the volume of alliin solution; Vf is the final volume made (*i.e.* 2 mL), and the factor 2 was used as two molecules of alliin were required to produce one molecule of allicin.

2.3. Method of extraction

The *Allium* leaf sample (approximately 100 g) was chopped and randomly crushed in an electric blender. From this crushed material, 10 ± 0.1 g was weighed out and mixed with 40 g water, and followed the extraction procedure reported by Khar et al. (2011) for garlic bulbs with minor modification. Following the procedure, the final supernatant was filtered through 0.2 μm N66 membrane (PALL Life Sciences, India), and injected (10 μl) into the LC-MS/MS system (API 5500 Q-trap LC–MS/MS, AB Sciex, Canada).

2.4. Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis

LC-MS/MS analysis was carried out using electrospray ionization (ESI) in positive polarity and multiple reaction monitoring (MRM) using the mass parameters optimized previously (Khar et al., 2011) as given in Table 1. The source parameters were set as follows: ion source voltage (5500 V), nebulizer gas (40 psi), heater gas (60 psi), and ion source temperature (500 ˚C). The chromatographic separation of the test compounds was achieved on Atlantis T3 (μm, 100 × 2.1 mm; Waters Corporation, Milford, Massachusetts, USA) HPLC column. Mobile phases were water (100%) as phase A and methanol (100%) as phase B in a gradient run of 16 min with programme: 0–1 min 15% B, 1–3 min 15–98% B, 3–11 min 98% B, 11–12 min 98-15% B, and 12–16 min 15% B. The column oven temperature was maintained at 40 (± 1)°C with mobile phase flow rate of 0.4 mL/min and injection volume of 10 μl. LC-MS/MS chromatogram of a *Allium* leaf sample is given in Fig. 1 indicating the peaks of Allicin (RT 9.56 min), AMThs (RT 8.31 min), and ATPThs (RT 10.07 min). Quantitative estimation of all the allyl thiosulphinates (Allicin, AMThs, and ATPThs) was done against allicin calibration standards.

2.5. Method validation

The analytical method was validated as per SANTE/11813/2017 guideline for allicin analysis with respect to linearity, limit of detection (LOD), limit of quantification (LOQ), and precision. Linearity of five point calibration curve was established at a calibration range of 1.0–50.0 μg/mL. The limit of detection (LOD) and the limits of quantification (LOQ) of the target compound was set at signal to noise (S/N) of 3 and 10, respectively. Inter-day ($n = 18$) and intra-day ($n = 6$) precision was estimated in terms of repeatability expressed as % RSD.

2.6. Estimation of dry weight of each accession

To measure the dry weight of each accession, approximately 10 g of the finely chopped fresh leaf sample were dried in a hot-air oven at
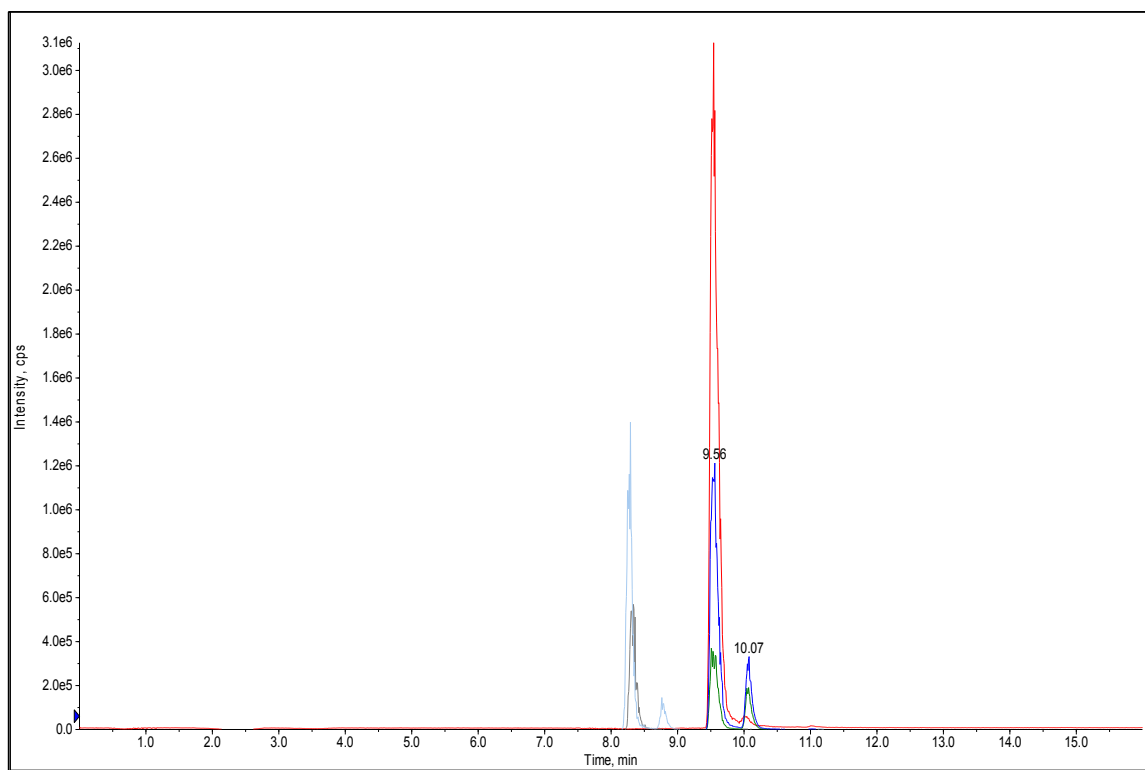
Fig. 1. LC-MS/MS chromatogram of an *Allium* leaf sample indicating the chromatographic separation of allicin (RT 9.56 min), AMThs (RT 8.31 min), and ATPThs (RT 10.07 min).

60 °C ($n = 3$), and dry weight of each sample were measured.

2.7. Data analysis

The data on allyl thiosulphinates were subjected to analysis of variance (ANOVA) using a software package, namely Statistical Analysis System (SAS) ver. 9.3. Significant differences, if any, between the means of the groups based on the degree of domestication and on leaf type were ascertained at a *p* value ≤ 0.05 (5% level of significance) using the PROC GLM procedure, and the mean values were compared using the least significant difference (LSD) test. The data were also subjected to cluster analysis and principal component analysis (PCA) by R packages, cluster and GGEBiplot GUI. Hierarchical clustering was used for identifying groups in given data-set. Mean or average linkage clustering method was used to measure the dissimilarity between two clusters. First the dissimilarity values with R function dist (Euclidean distance measures used) was computed and then these values were fed into R function hclust, and mean or average linkage clustering agglomeration method was used for clustering. A multivariate statistical analysis GGE biplot was created using GGEBiplot GUI package in *R* to understand the relationship between functional constituents and genotypes.

3. Results and discussions

3.1. Validation of the method of analysis

The analytical method used for extraction and analysis of allicin was validated as per the standard method performance requirements of SANTE/11813/2017. The linearity of calibration curve was established within a range of 1.0–50 µg/mL with $r^2 = 0.9952$. Based on the signal to noise ratio (S/N), the LOD for allicin was 1.0 µg/mL and the LOQ was 3.0 µg/g. A satisfactory intra-laboratory precision was confirmed since

the RSD was less than 10% in intra-day ($n = 6$) and inter-day ($n = 18$) assays.

3.2. Allicin, allyl methyl thiosulphinate, and allyl trans-1-propenyl thiosulphinate in different Allium species

Mean concentrations of individual thiosulphinates (allicin, AMThs, and ATPThs) in different *Allium* accessions are shown in Table 2 on both fresh weight (fw) and dry weight (dw) basis. On fresh weight basis, allicin content in these accessions ranged from 0.00 ppm (in *A. altaicum*, *A. ampeloprasum*, *A. ascalonicum*, *A. cepa*, *A. chinense*, *A. fistulosum*, *A. fragrance*, *A. ledebourianum*, and *A. schoenoprasum*) to 344.00 ppm in *A. sativum* (Table 2). On dw basis the corresponding concentrations were 0.00 and 2941.94 ppm. The differences were statistically significant ($p \leq 0.05$). The highest content was recorded in accession ASa25 followed by ATu31, AGu23, and ATu29 (Table 2). These results are consistent with an earlier report (Block et al., 1992) on allicin and other thiosulphinates content in many *Allium* species. Allicin was also reported in A. tuberosum Rottler and A. chinense G. Don (Rakkyo) as an intermediate compound during the formation of dithiins (Yabuki et al., 2010).

The levels of AMThs were within a broad range of 0.00–353.00 ppm (fresh weight) and 0.00–3549.50 ppm (dw), and the differences in AMThs content among *Allium* accessions were significant. The levels were low (0.00–364.07 ppm) in *A. altaicum*, *A. ampeloprasum*, *A. ascalonicum*, *A. cepa*, *A. chinense*, *A. fasciculatum*, *A. fistulosum*, *A. ledebourianum*, *A. sativum*, and *A. schoenoprasum*; high (2190.93–3448.96 ppm) in *A. guttatum*, *A. tuberosum*, and Zimmu; and AMThs were not detected at all in *A. fragrance*. In some cases, AMThs content varied within accessions of the same species as well.

The levels of ATPThs were within a range of 0.00–46.40 ppm (fresh weight) and 0.00–396.82 ppm (dw), the highest value being in *A. sativum*. Fairly high levels (26.47–157.36 ppm) were recorded in *A.*

Table 2

List of *Allium* accessions, code and amount of allicin, allyl methyl thiosulphinates (AMTHs), and allyl trans-1-propenyl thiosulphinates (ATPThs).

Code	Name of collection	Allicin	AMTHs	ATPThs	Allicin	AMTHs	ATPThs
		Fresh weight basis fw (ppm)			Dry weight basis dw (ppm)		
AAl01	*A. altaicum* pall. (Ausdauernd)	ND^j	0.82 ± 0.01^{no}	0.78 ± 0.03^m	ND^j	6.09 ± 0.11^s	5.80 ± 0.19^q
AAl02	*A. altaicum* pall.	ND^j	0.45 ± 0.03^{no}	0.60 ± 0.08^{mn}	ND^j	4.16 ± 0.22^{tu}	5.49 ± 0.22^q
AAl03	*A. altaicum* pall.	ND^j	3.15 ± 0.10^{jk}	1.41 ± 0.06^k	ND^j	25.35 ± 0.47^m	11.35 ± 0.15^m
AAl04	*A. altaicum* pall. (Mangolei)	ND^j	ND^o	0.74 ± 0.05^m	ND^j	ND^v	6.34 ± 0.41^{op}
AAl05	*A. altaicum* pall.	ND^j	ND^o	ND^o	ND^j	ND^v	ND^s
AAm06	*A. ampeloprasum*	ND^j	0.61 ± 0.02^{no}	0.83 ± 0.01^m	ND^j	4.58 ± 0.09^t	6.27 ± 0.01^{op}
AAm07	*A. ampeloprasum*	ND^j	ND^o	0.58 ± 0.03^{mn}	ND^j	ND^v	6.80 ± 0.13^o
AAs08	*A. ascalonicum* (Pran1)	ND^j	2.39 ± 0.03^{kl}	2.36 ± 0.13^j	ND^j	26.81 ± 1.32^l	26.47 ± 0.40^l
AAs09	*A. ascalonicum* (Pran2)	ND^j	3.51 ± 0.07^j	5.42 ± 0.37^e	ND^j	33.37 ± 0.09^k	51.52 ± 0.18^f
ACe10	*A. cepa* L. var. *aggregatum*-5	ND^j	2.54 ± 0.08^{kl}	ND^o	ND^j	21.32 ± 0.39^n	ND^s
ACe11	*A. cepa* L. var. *aggregatum*-4	ND^j	2.09 ± 0.14^{lm}	ND^o	ND^j	19.92 ± 0.42^o	ND^s
ACe12	*A. cepa* L. var. *aggregatum*-3	ND^j	1.18 ± 0.13^{mn}	1.10 ± 0.21^l	ND^j	10.04 ± 0.09^q	9.36 ± 0.28^n
ACe13	*A. cepa* L. (Uzbekistan)	ND^j	0.34 ± 0.01^{no}	ND^o	ND^j	3.26 ± 0.35^u	ND^s
ACe14	*A. cepa* L. (common onion)	ND^j	0.53 ± 0.02^{no}	ND^o	ND^j	5.68 ± 0.30^s	ND^s
ACh15	*A. chinense* (Rakkyo)	ND^j	2.18 ± 0.04^l	ND^o	ND^j	18.09 ± 0.32^p	ND^s
AFa16	*A. fasciculatum*	5.80 ± 0.71^h	27.30 ± 0.85^h	11.80 ± 0.27^b	77.35 ± 0.63^h	364.07 ± 0.32^h	157.36 ± 0.76^b
AFi17	*A. fistulosum* L. (Georgia)	ND^j	0.43 ± 0.02^{no}	ND^o	ND^j	3.65 ± 0.17^u	ND^s
AFi18	*A. fistulosum* L. (Taiwan)	ND^j	ND^o	ND^o	ND^j	ND^v	ND^s
AFi19	*A. fistulosum* (China)	ND^j	ND^o	ND^o	ND^j	ND^v	ND^s
AFi20	*A. fistulosum*	ND^j	0.95 ± 0.01^n	ND^o	ND^j	8.52 ± 0.56^r	ND^s
AFi21	*A. fistulosum*	ND^j	ND^o	ND^o	ND^j	ND^v	ND^s
AFr22	*A. fragrance*	ND^j	ND^o	ND^o	ND^j	ND^v	ND^s
AGu23	*A. guttatum*	31.80 ± 1.27^c	336 ± 0.37^b	3.10 ± 0.17^i	326.42 ± 0.42^c	3448.96 ± 0.57^b	31.82 ± 0.21^j
ALe24	*A. ledebourianum*	ND^j	0.98 ± 0.01^n	0.59 ± 0.04^{mn}	ND^j	10.70 ± 0.40^q	6.47 ± 0.22^{op}
ASa25	*Allium sativum* L. (common garlic)	344 ± 1.56^a	27.90 ± 0.57^h	46.40 ± 0.18^a	2941.94 ± 0.19^a	238.60 ± 0.52^i	396.82 ± 0.28^a
ASc26	*A. schoenoprasum*	1.55 ± 0.10^i	21.60 ± 0.28^i	7.98 ± 0.21^c	13.29 ± 0.44^i	185.20 ± 0.20^i	68.42 ± 0.31^d
ASc27	*A. schoenoprasum*	ND^j	1.96 ± 0.16^{lm}	0.47 ± 0.10^n	ND^j	17.66 ± 0.35^p	4.21 ± 0.31^r
ATu28	*A. tuberosum*	16.60 ± 0.57^g	269 ± 0.99^d	3.93 ± 0.08^h	182.96 ± 0.92^f	2964.89 ± 0.43^d	43.32 ± 0.21^i
ATu29	*A. tuberosum* Hanzhong winter	22.30 ± 1.13^e	256 ± 0.42^e	4.38 ± 0.17^g	245.79 ± 0.26^d	2821.60 ± 0.49^e	48.28 ± 0.15^h
ATu30	*A. tuberosum* Rottler ex Spr.	23.50 ± 0.99^d	210 ± 1.41^g	5.39 ± 0.03^e	245.18 ± 0.42^d	2190.93 ± 1.23^g	56.23 ± 0.31^e
ATu31	*A. tuberosum* Bawang kucai	32.80 ± 0.57^b	353 ± 0.57^a	3.06 ± 0.14^i	329.81 ± 0.86^b	3549.50 ± 0.70^a	30.77 ± 0.23^k
ATu32	*A. tuberosum* Rottl. ex spr.	18.30 ± 0.14^f	299 ± 1.13^c	6.85 ± 0.11^d	201.70 ± 1.02^e	3295.54 ± 0.64^c	75.50 ± 0.06^c
Zim33	Zimmu (*Allium cepa* L. × *Allium sativum* L.)	16.90 ± 0.57^g	226 ± 0.85^f	4.66 ± 0.24^f	181.76 ± 0.20^g	2430.58 ± 0.16^f	50.12 ± 0.17^g

ND, Not detected.

Experimental values are expressed as mean $\pm$ SD ($n = 3$).

Mean followed by the same superscripts are not significantly different ($p \leq 0.05$).

ascalonicum, *A. fasciculatum*, *A. schoenoprasum*, *A. tuberosum*, and Zimmu whereas ATPThs were not detected at all in a few cases: *A. altaicum* (AAl05), *A. cepa* (ACe10, ACe11, ACe13, ACe14), *A. chinense* (ACh15), *A. fistulosum* (AFi17, AFi18, AFi19, AFi20, AFi21), and *A. fragrance* (AFr22).

The highest amounts of total thiosulphinates were recorded in *A. sativum*, followed by *A. guttatum*, A. tuberosum, and Zimmu and may account for their antimicrobial activity (Mylona et al., 2019) and for their role as a food preservative (Mellado-García et al., 2015). On both fw and dw basis, allicin accounted for 0%–12.9% of the total thiosulphinates in all the genotypes except *A. sativum* (allicin content of which was 82%). In most genotypes, AMTHs levels were 40%–100%; ATPThs levels were usually low (0%–11%) but more than 20% in a few accessions.

To date, only a few investigations on *Allium* species have included thiosulphinates: *A. cepa* (Block et al., 1992; Calvey et al., 1997); *A. sativum* (Gonzalez et al., 2009; Khar et al., 2011; Sterling and Eagling, 2001); *A. fistulosum* (Block et al., 1992; Kuo and Ho, 1992); *A chinense* (Rakkyo) (Yabuki et al., 2010); *A. ampeloprasum*, *A. schoenoprasum*, and *A. ascalonicum* (Block et al., 1992); and *A. tuberosum* (Block et al., 1992; Kim et al., 2008; Yabuki et al., 2010). There are no reports on thiosulphinate content of *A. altaicum*, *A. fasciculatum*, *A. guttatum*, *A. ledebourianum*, and Zimmu.

Thiosulphinates are commercially important for their health benefits. Use of extracts and compounds from *Allium* as antimicrobial (antibacterial and antifungal) agents and food preservatives – preferred as a natural alternative to synthetic preservatives – is common, and some products have even been awarded patents (Lara-Cambil and Pareja, 2007; Heras-Mozos et al., 2019). According to the British herbal

pharmacopoeia, the minimum allicin content to ensure pharmaceutical and commercial viability should be 4.5 mg/g in garlic powder products (Avato et al., 1998). Allicin content in garlic leaves in the present experiment (0.013–2.941 mg/g dw) was less than that in garlic bulbs (5.03 and 18.24 mg/g dw; Khar et al., 2011) and less than this standard value but, by taking all types of analysed thiosulphinates together, many species were found to be quite rich and comparable to the British herbal pharmacopoeia standard: *A. guttatum* (3.808 mg/g), *A. sativum* (3.578 mg/g), *A. tuberosum* (3.911 mg/g), and Zimmu (2.662 mg/g). Because *A. guttatum*, *A. tuberosum*, and Zimmu can be harvested every month throughout the year, the annual yield of thiosulphinate will be even higher. These plants will provide a year-round supply of material. *Allium sativum*, *A. guttatum*, *A. tuberosum*, and Zimmu are the richest sources of total thiosulphinates and can be used as a potential source for extracting thiosulphinates and also in making different preservative formulations (Mellado-García et al., 2016; Raeisi et al., 2016; Snoussi et al., 2016).

3.3. Allicin and other allyl thiosulphinates content based on leaf type

Leaf type had a significant effect ($p \leq 0.05$) on allicin, AMTHs, ATPThs, and total thiosulphinate content (Table 3). Accessions with flat and non-waxy leaves recorded higher levels of mean thiosulphinates (allicin, 430.26 ppm; AMTHs, 1938.43 ppm; ATPThs, 80.93 ppm; total: 2449.63 ppm) than those with fistular and waxy leaves (allicin, 0.60 ppm; AMTHs, 17.56 ppm; ATPThs, 9.48 ppm; total: 27.64 ppm). To our knowledge, the link between leaf type and allicin and other allyl thiosulphinates content has not been reported so far; therefore, the results cannot be compared with those from any earlier study. However,

Table 3
Mean values of allicin, allyl methyl thiosulphinates (AMThs), allyl trans-1-propenyl thiosulphinates (ATPThs), and total thiosulphinates in *Allium* accessions based on foliage type.

Foliage type	Allicin Fresh weight basis fw (ppm)	AMThs	ATPThs	Total thiosulphinates	Allicin Dry weight basis dw (ppm)	AMThs	ATPThs	Total thiosulphinates
(Fistular and waxy)[20]	0.07	1.98	1.04	3.09	0.60	17.56	9.48	27.64
(Flat and non-waxy)[13]	46.55	182.40	8.14	237.09	430.26	1938.43	80.93	2449.63
LSD (0.05)	42.46	59.88	5.73	65.64	359.61	630.17	49.56	648.11
GLM procedure ($Pr > F$)	*	*	*	*	*	*	*	*

Significance of $p \leq 0.05$.

Table 4
Mean values of allicin, allyl methyl thiosulphinates (AMThs), allyl trans-1-propenyl thiosulphinates (ATPThs), and total thiosulphinates in *Allium* accessions based on cultivation status.

Cultivation status	Allicin Fresh weight basis fw (ppm)	AMThs	ATPThs	Total thiosulphinates	Allicin Dry weight basis dw (ppm)	AMThs	ATPThs	Total thiosulphinates
(Wild)[9]	0.00	0.92	0.43	1.35	0.00	8.05	3.70	11.75
(Land race)[4]	0.00	0.19	0.19	0.38	0.00	1.73	1.58	3.31
(Semi-domesticated)[14]	12.11	143.28	4.25	159.65	128.88	1523.54	46.19	1698.60
(Cultivated)[6]	57.33	5.81	8.06	71.20	490.32	50.02	68.74	609.09
LSD (0.05)	61.70	108.89	9.07	142.03	566.61	1148.6	80.48	1415.40
GLM Procedure ($Pr > F$)	ns	*	ns	*	ns	*	ns	*

Significance of $p \leq 0.05$.
Here, ns indicates not significant.

leaf chemistry has been correlated to leaf morphology (Sytara et al., 2018) and wax content (Schneider et al., 2016). Leaf morphology (Li et al., 2018), wax content, and leaf chemistry (Singh et al., 2018) play a major role in tolerance to biotic and abiotic sources of stress (Guo et al., 2016) which, in turn, may affect the degree of domestication.

3.4. Allicin and other allyl thiosulphinates content based on degree of domestication

In general, cultivated and semi-domesticated accessions recorded higher total thiosulphinate content than wild types and landraces (Table 4) although the differences were significant only for AMThs and total thiosulphinates and not for allicin and ATPThs. Wild types and landraces showed very low levels of allicin, AMThs, ATPThs, and total thiosulphinates. The highest mean values of allicin (490.32 ppm) and ATPThs (68.74 ppm) were recorded in cultivated species whereas those of AMThs (1523.54 ppm) and total thiosulphinates (1698.60 ppm) were recorded in semi-domesticated types. Thiosulphinate compounds therefore appear to be positively linked to the degree of domestication: the higher the thiosulphinate content, the greater the degree of domestication and, in turn, the greater the preference for cultivation. Species with higher amounts of thiosulphinate compounds are more commonly cultivated because of their health benefits and greater tolerance to biotic and abiotic forms of stress. These results are consistent with the findings of Won et al. (2017); Gonzalez et al. (2009), and Lawson et al. (1991a, 1991b), who also reported that domestication and cultivation are influenced by the biological importance and adaptation.

3.5. Cluster analysis

All the 33 accessions were clustered into three groups (Fig. 2A). The first group included 25; the second, 7; and the third, only 1. The first group comprised the following accessions: AAl01, AAl02, AAl03, AAl04, AAl05, AAm06, AAm07, AAs08, AAs09, ACe10, ACe11, ACe12, ACe13, ACe14, ACh15, AFa16, AFi17, AFi18, AFi19, AFi20, AFi21, AFr22, ALe24, ASc26, and ASc27. All the genotypes in this group had variable amounts of allicin (0–77.35 ppm), AMThs (0–364.07 ppm), and ATPThs (0–157.36 ppm) and most of them had fistular and waxy leaves except AAm06, AAm07, ACh15, AFa16, and AFr22, which had

flat and non-waxy leaves. The second group mainly covered the accessions with flat and non-waxy leaves (AGu23, ATu28, ATu29, ATu30, ATu31, ATu32, and Zim33), all of which were rich in allicin (181.76–329.81 ppm) and ATPThs (30.77–75.50 ppm) and showed very high levels of AMThs (2190.93–3549.50 ppm). The third group included only ASa25 (*A. sativum*), which had the highest amounts of allicin and ATPThs. The clustering obtained in the present experiment is consistent with that obtained by Kumar et al. (2018) and Koley et al. (2014), who used cluster analysis for establishing phylogenetic relationships based on morphological and biochemical traits.

The degree of domestication showed no significant correlation with clustering. Members of the first group represented all the four categories based on the degree of domestication: wild types (AAl01, AAl02, AAl03, AAl05, AFi18, AFi20, AFr22, ALe24, and ASc27); landraces (AAl04, ACe13, AFi17, and AFi19), semi-domesticated (AAm07, AAs08, AAs09, ACh15, AFa16, AFi21, and ASc26); and cultivated (AAm06, ACe10, ACe11, ACe12, and ACe14). Members of the second group (AGu23, ATu28, ATu29, ATu30, ATu31, ATu32 and Zim33) were all in the semi-domesticated category, and the sole member of the third group (ASa25) belonged to the cultivated category.

The biplot ranked all the 33 accessions by the extent of variation in allyl thiosulphinates. Principal component analysis of six quantitative traits (allicin, AMThs, and ATPThs, each on fw and dw basis) revealed that two principal components explained 99.95% of the variation: principal component 1 explained 86.78%; principal component 2, 13.17% (Fig. 2B). Genotype ASa25 recorded the highest allicin content; genotypes AGu23, ATu28, ATu29, ATu30, ATu31, and ATu32 also had good amounts of AMThs.

4. Conclusions

Most of the work so far on thiosulphinates has focused on garlic and other edible species of *Allium*: information on wild species is meagre. *Allium* genotypes examined in the present study – representing both wild and cultivated *Allium* – differed significantly in their thiosulphinates content. The information obtained in the present study can help in better utilization of other *Allium* species as well for pharmaceutical uses. Because *A. sativum*, *A. guttatum*, *A. tuberosum*, and Zimmu (an interspecific hybrid of *Allium cepa* L. and *Allium sativum* L.) were the
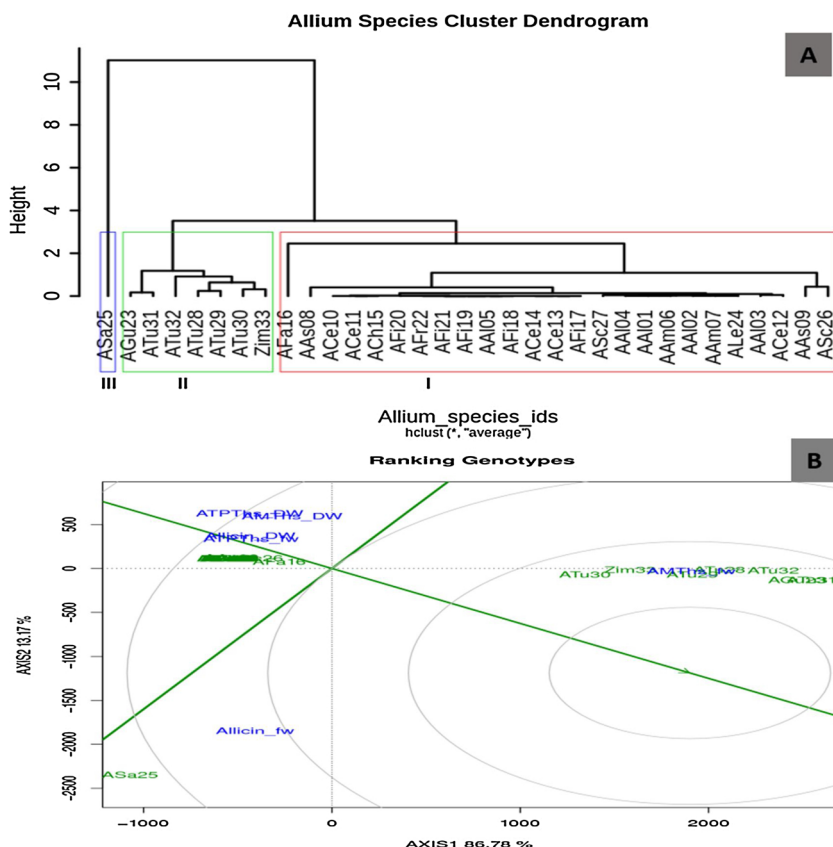
Fig. 2. Cluster analysis of *Allium* accessions based on allicin and other allyl thiosulphinates by hierarchical cluster analysis using R packages, cluster and GGEBiplot GUI. (A) *Allium* accessions cluster dendrogram by hclust R function using mean or average clustering agglomeration method. Here all the *Allium* accessions are divided into three significantly different clusters which are illustrated in results and discussions; (B) Cluster biplot of first and second principal component based on the variation of allyl thiosulphinates in *Allium* accessions using GGEBiplot GUI package in R.

richest in total thiosulphinates, they are the best choice for commercial cultivation. The second and the third clusters had as their members those species that had the highest levels of thiosulphinate, and all of them belong to the cultivated and semi-domesticated types. Many of the species are edible, and can be included in breeding for high thiosulphinates content. Leaves of the more promising *Allium* accessions could be used as food, a source of medicines, and a natural and non-toxic food preservative.

Conflict of interest

The authors declare that there is no conflict of interest.

Credit author statement

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2020.112215.

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