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Rapid GC-MS based metabolic profiling of *Capsicum annuum* L. seeds at different phases of germination

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Abstract

Seed germination is the most critical stage of the life cycle of a plant. Metabolic regulation is significant during the course of seed germination for the establishment of seedlings. Upon imbibition, the dry and fully developed seeds undergo the release of various organic molecules, such as low molecular weight carbonyl compounds in the form of gases and volatiles, as well as water-soluble organic components like enzymes and polysaccharides. Volatile organic compounds may impart both positive and negative influences on seed germination. A metabolite profiling approach based on gas chromatography–mass spectrometry (GC-MS) was used to investigate time-dependent metabolic changes during the germination of *Capsicum annuum* L. cv. 'Bullet'. This study aimed to identify bioactive compounds from the methanolic extract of dry, imbibed, germinating seeds and young seedlings of the 'Bullet' cultivar of *C. annuum* L. by GC-MS. A total of 56 distinct categories of compounds were identified in dry seeds, while fully absorbed seeds contained 53 compounds. In the germinating seed, 52 compounds were identified. With regard to immature seedlings, a total of 28 compounds were identified. The analysis revealed that each stage has a unique bio-constituent; only five compounds were detected in all extracts.

Keywords: *Capsicum annuum* cv. 'Bullet'; GC-MS; imbibition; seed germination; secondary metabolites

Introduction

The seed germination stage is one of the essential developmental stages of plant life during which numerical interactions of biochemical molecules take place. The proper germination of a seed necessitates a systematic relationship between the embryo, seed coat, and intermediate endosperm (Sarkar and Sadhukhan, 2022). In the context of crop production, achieving optimal seed germination and successful seedling establishment is a highly sought-after characteristic by farmers, as it contributes to the long-term viability and financial gains of their agricultural endeavors. The process of germination is a multifaceted phenomenon in

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which the seed undergoes physical changes that enable it to recover from desiccation, engage in sustained metabolic activity, accomplish crucial cellular processes necessary for the emergence of the embryo, and overall adapt its entire metabolic state to facilitate subsequent seedling growth (Nonogaki *et al*., 2010). Upon imbibition, the dry and fully developed seeds undergo the release of various organic molecules, such as low molecular weight carbonyl compounds in the form of gases and volatiles, as well as water-soluble organic components like enzymes and polysaccharides. The seed germination stage is characterized by a mixture of catabolic and anabolic processes. Most plant species produce volatile chemicals in their seeds when they germinate, and these compounds are usually produced at a high rate in the first three to four days after imbibition. The nature and emission dynamics of volatiles produced by seeds differ according to their moisture content (Umarani *et al*., 2020). The moisture content of the seed is a significant regulator of biochemical processes, and it determines the nature and emission kinetics of volatile substances produced by seeds. The germination and growth processes of seeds often encompass a range of metabolic alterations, including modifications in the nutritional composition of seeds, particularly in terms of proteins and carbohydrates (Sarkar and Sadhukhan, 2022). These changes serve to furnish the seeds with the necessary energy for successful germination and subsequent growth. Several studies unequivocally demonstrated the effect of volatile organic compound emission levels on seeds' physiological and biochemical characteristics during germination and early seedling development (Chinnasamy *et al*., 2022). However, there is a dearth of information on the volatile chemicals and their potential.

Investigating volatiles linked to seed germination and post-germination development is an area of research that has received little attention. To the best of our understanding, there is currently a lack of scientific discourse about the relation of volatiles and seed germination of *Capsicum annuum* L. cv. 'Bullet'. 'Bullet' chilli is one of the famous cultivars of *C. annuum* L., widely cultivated in the Indian subcontinent (Sarkar *et al*., 2023). The seed germination of the cultivar is somewhat resistant to biotic and abiotic stresses and is usually cultivated throughout the year. Therefore, it is crucial to investigate the phytochemicals found in 'Bullet' chilli seeds at different stages of germination and their possible applications in agriculture and biology. This research is also useful for understanding the role of these chemicals in seed germination and the promotion of the thriving crop.

GC-MS is particularly valuable for obtaining a relative quantitation of critical metabolites involved in plant primary metabolism, including amino acids, small soluble sugars, polyamines, and organic acids, within a single sample analysis. It was utilized as a functional instrument for simultaneous analysis of phytoconstituents present in the seed extracts (Palekar *et al*., 2020). After solvent extraction and derivatization, it is possible to characterize hundreds of metabolites from distinct chemical classes in a single analytical sample. GC-MS can quickly determine volatiles and gases produced by plant tissues. Volatiles represent a minor portion of the total substances produced by organisms, but their unique properties allow them to mediate essential biological functions, particularly in aerial and terrestrial environments (Mihaylova *et al*., 2022). There are many methodologies for analyzing volatile substances using GC-MS. The process of static headspace analysis entails the containment of the sample within a sealed container, followed by the measurement of the volatile substances released. Direct headspace sampling can be achieved using a gas-tight syringe (Mira *et al*., 2010). The concentration of volatiles can be achieved by utilizing an adsorbent trap, such as Tenax, which is a polymer resin (Zhang *et al*., 1993). Solid phase microextraction (SPME) uses an adsorbent fibre within a syringe. The fibre is subjected to the gaseous environment present in a vial, and the volatile molecules that become trapped are then transported to a gas chromatography-mass spectrometry (GC-MS) system by thermal desorption (Mira *et al*., 2010). As mentioned above, the sample uses strategies previously employed in investigations pertaining to seed volatiles.

However, there is a scarcity of literature that compares these methodologies. Most GC-MS-based studies on germinating seeds have focused on the nutritional properties of edible germinating seeds, but GC-MS studies are scarce with respect to physiological aspects. Some of the GC-MS studies showed the presence of

several primary (carbohydrates, amino acids, organic acids, and lipids) and secondary (phenolic acids) metabolites that have positive effects on the physiological activities of plant tissues (Mihaylova *et al*., 2022). The 'Bullet' cultivar of *C. annuum* L. is appreciated as a Bengal landrace as it is widely cultivated in West Bengal and adjoining regions of India (Sarkar *et al*., 2023). The cultivar is highly demanding for its nutritional value and low production cost. *C. annuum* L. is a highly valuable vegetable crop with significant commercial value in international trade (Sarkar and Sadhukhan, 2023). Several cultivars of the species are cultivated in several agricultural regions of the globe throughout the year, whereas some cultivars are specific to particular environmental circumstances (Rao and Anilkumar, 2020). Some cultivars are significantly affected by biotic and abiotic stresses throughout their lifecycle, most significantly during seed germination. Germination is associated with systematic and synchronized biochemical and metabolic processes (Sarkar and Sadhukhan, 2022). Such compounds can either induce or suppress seed dormancy due to the influence of abiotic influences on organic components present in seeds (Motsa *et al.*, 2017). Volatile compounds play an essential part in the induction as well as in the maintenance of seed dormancy (Hung *et al*., 2014). Metabolite profiling is considered an effective analytical approach to comprehending these changes entirely. The combination of gas chromatography and mass spectrometry has shown to be a highly successful approach for chemical analysis and metabolite profiling (Krishnaveni *et al*., 2014). It is a fact that many environmental as well as genetic factors contribute to the chemical and biological differences between cultivars, generating scientific curiosity for further investigation of the plant metabolome.

Materials and Methods

Seed germination

The viable seeds of *Capsicum annuum* cv. 'Bullet' were utilized to conduct a seed germination bioassay. The seeds underwent surface sterilization using a 0.02% aqueous solution of mercuric chloride for a duration of 2 minutes. Following this, the seeds were rinsed extensively with distilled water. Subsequently, 30 seeds were placed on individually sterilized Petri plates with a 10 cm diameter lined with filter paper. 5 ml of distilled water was applied to each Petri plate. The treatment was placed in a growth chamber maintaining 65-80% relative humidity, 28 ± 2 °C temperature and a 16:8 light/dark cycle with 3 replications. Every Petri plate was properly irrigated with distilled water and monitored for 10 days.

GC-MS analysis of sample

One of the most reliable approaches for the comprehensive study of major plant metabolites is the combination of capillary gas chromatography and mass spectrometry (GC-MS), which has been established among many technological platforms for metabolite profiling (Khakimov *et al*., 2017). Global metabolomics techniques often employ extraction mixes encompassing a broad spectrum of metabolites, ranging from polar to apolar. Examples of such combinations are H_2O: MeOH: $CHCl_3$ (1:2.5:1) or H_2O: MeOH, which are commonly preferred (Rohloff, 2015). The GC-MS Analysis of the Sample was performed at Aakaar Biotechnologies Private Limited, Lucknow, India. 500 µl of the sample (1 mg/ml) was taken in a separating funnel and shaken by adding 10 ml of water and ethyl acetate in a ratio of 1:4 (add 2.5 ml water to 7.5 ml Ethyl Acetate). The upper layer was collected and concentrated to 1 ml in the rotary evaporator. 50 µl N, O-Bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane (BSTFA+TMCS) were added and then finally 10 µl of Pyridine was also added. Samples were further heated at 60 °C for 30 minutes. For BSTFA+TMCS, make 100 µl solution of 99 µl of BSTFA and 1µl of TMCS. Samples were transferred in a GC vial and dried using nitrogen gas. Finally, samples were dissolved in methanol before GC-MS analysis. The instrument used in this study is GC-2010 Plus (Shimadzu Europa GmbH). Acquired samples were programmed as described in the supplementary data.

The data were plotted in 'jvenn' online software (Bardou *et al*., 2014) to compare the identified compounds in GC-MS of dry seeds and different developmental stages of *C. annuum* cv. 'Bullet'.

Results

The different stages of germination of *C. annuum* cv. 'Bullet' seeds, i.e., dry, imbibed, and germinated seeds and young seedlings (Figure 1A, B, C and D, respectively), were used in this study. The GC-MS analysis demonstrated the existence of many primary and secondary metabolites that significantly impact the germination process and subsequent development of seedlings. 122 compounds were found and identified in germinating seeds of 'Bullet' chilli. These phytochemicals are depicted in Table 1 and Figure 2 (A, B, C, D), the chromatogram of dry, imbibed, and germinated seeds and young seedlings, respectively. During distinct seed germination phases, these constituents' chemical properties vary considerably.

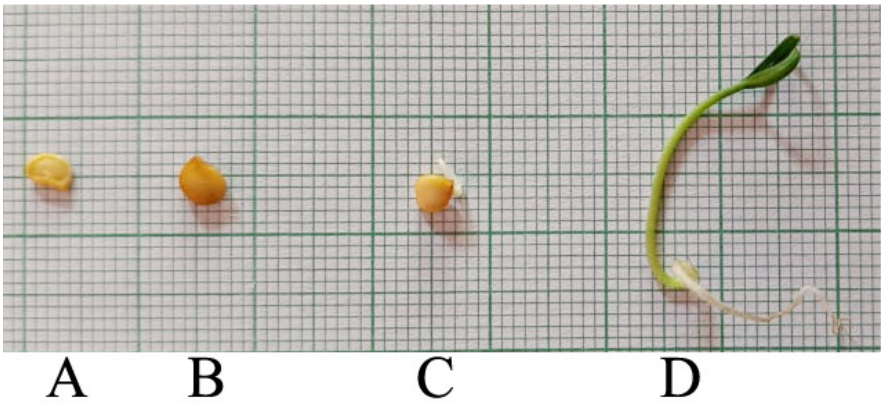

Figure 1. Dry, imbibed, germinating seed and young seedling used in the experiment

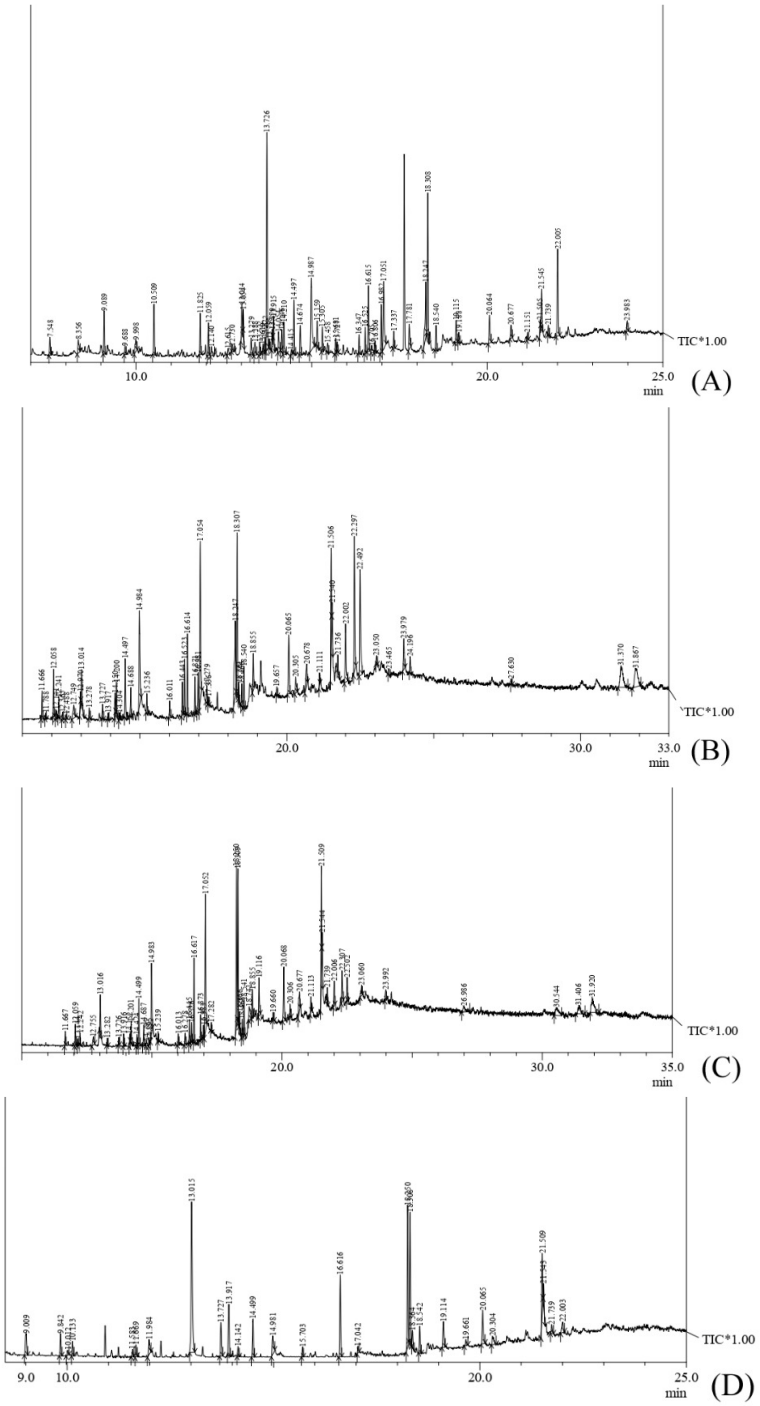

Figure 2. Chromatogram of different metabolites obtained from (A) dry, (B) imbibed, (C) germinating seeds and (D) young seedlings

Table 1. Comparison between the identified compounds based on their peak area (%) in GC-MS study of dry seed and different growth stages of *C. annuum* cv. 'Bullet'

Sl No.	Compound	Dry seed	Imbibed seed	Germinating seed	Young seedlings
1	Nonane, 3,7-Dimethyl	1.02	-	-	-
2	1,5-Dimethyl-1-Vinyl-4-Hexenyl Butyrate	1.10	-	-	-
3	Tetradecane	2.73	1.67	-	-
4	P-(Methoxymethyl)-Isopropylbenzene	0.51	-	-	-
5	Benzenepropionic Acid, 4-Tetradecyl Ester	1.10	-	-	-
6	Heptadecane	2.07	-	-	-
7	2,4-Di-Tert-Butylphenol	1.55	-	-	-
8	Nonanoic Acid, 7-Methyl-, Methyl Ester	0.59	0.42	-	-
9	Docosane	0.24	0.32	0.82	-
10	Cyclohexanecarboxylic Acid, Cyclohexyl Ester	0.47	-	-	-
11	Diethyl Phthalate	1.62	-	-	19.63
12	Benzene, 1,2,4-Trimethoxy-5-(1-Propenyl)	1.14	-	-	-
13	2-Methylcyclohexyl Ethylphosphonofluoridate	0.94	-	-	-
14	2-(4a,8-Dimethyl-2,3,4,5,6,7-Hexahydro-1H-Naphthalen-2-yl)propan-2-ol	0.70	-	-	-
15	2-Ethoxy-4-Methyl-1-Phenacylbenzimidazol	0.39	-	-	-
16	2-((2R,8R,8as)-8,8a-Dimethyl-1,2,3,4,6,7,8,8a-Octahydron	0.72	-	-	-
17	Methyl (3-Oxo-2-Pentylcyclopentyl) Acetate	12.65	0.67	-	-
18	2-(4a,8-Dimethyl-2,3,4,5,6,7-Hexahydro-1H-Naphthalen-2-yl) propan-2-ol	0.39	-	-	-
19	(7a-Isopropenyl-4,5-Dimethyloctahydroinden-4-Yl) Methanol	1.19	-	-	-
20	1,6-Methanonaphthalen-1(2h)-Ol, Octahydro-4,8a,9,9-Tetramethyl (1.Alpha.,4.Beta., 4a.Alpha.,6.Beta., 8a.Alpha.)	1.28	-	-	-
21	Heneicosane	2.04	-	-	-
22	(4as,5r,8s,8as)-8-Isopropyl-5-Methyl-3,4,4a,5,6	0.27	-	-	-
23	Methyl Tetradecanoate	2.77	-	-	-
24	3-Hexadecanol	1.73	-	-	-
25	Tetradecanoic Acid	6.31	5.64	5.62	2.52
26	Naphtho[2,1-B]Furan, Dodecahydro-3a,6,6,9a-Tetramethyl-	1.48	-	-	-
27	Sulfurous Acid, Hexyl Octyl Ester	1.26	-	-	-
28	2-Ethylhexyl Salicylate	0.58	-	-	-
29	3-Oxabicyclo[5.1.0]Octane, 5,5-Dimethyl-4-(3-M	0.73	-	-	-
30	1-Acetyl-4-Methyl-1,3-Dihydro-Pyrrole-2,2-D	0.59	-	-	-
31	Spiro[Cyclopentane-1,2'(1'h)-Quinoxaline]	0.99	-	-	-
32	7,9-Di-Tert-Butyl-1-Oxaspiro(4,5)Deca-6,9-Diene-2,8-Dione	1.14	1.95	-	-
33	Hexadecanoic Acid, Methyl Ester	3.39	2.98	4.42	7.33
34	Phosphoramidic Acid, Diethyl Ester	0.54	-	-	-
35	1,7-Hexadecadiene	0.68	-	-	-
36	1,2-Benzenedicarboxylic Acid, Dibutyl Ester	2.5	1.61	-	-
37	Hexadecanoic Acid	5.17	8.96	10.44	-
38	Valeric Acid, 2-Cyano-4-Methyl-, Ethyl Ester	1.69	-	-	-

39	14-Methyl-8-Hexadecyn-1-Ol	4.09	-	-	-
40	9-Octadecenoic Acid (Z)-, Methyl Ester	8.04	6.55	7.59	11.39
41	Octadecanoic Acid, Methyl Ester	1.35	1.69	2.72	3.59
42	9,11-Octadecadienoic Acid, Methyl Ester	1.36	-	-	-
43	Oxalic Acid, Butyl 6-Ethyloct-3-Yl Ester	0.54	-	-	-
44	Glycidyl Palmitate	1.96	3.33	0	3.68
45	9-Octadecenamide, (Z)	0.80	-	0.87	0
46	2-Hydroxy-3-[(9e)-9-Octadecenoyloxy]Propy	0.39	-	-	-
47	8-Tetradecyn-1-Ol	0.48	-	-	-
48	9-Octadecenoic Acid (Z)-, Oxiranylmethyl Ester	1.93	0.63	0.97	1.26
49	Bis(2-Ethylhexyl) Phthalate	5.25	3.54	-	-
50	13-Docosenamide	0.73	-	-	-
51	Octadecane	-	1.27	-	-
52	Phenol, 2,4-Bis(1,1-Dimethylethyl)-	-	1.78	-	-
53	Decane, 3-Ethyl-3-Methyl-	-	0.91	-	-
54	1-Octadecene	-	0.86	-	-
55	1,2-Benzenedicarboxylic Acid, Diethyl Este	-	1.57	2.32	-
56	1,2,4-Trimethoxy-5-[(1e)-1-Propenyl]Benzene	-	0.50	0.41	-
57	5-Chlorovaleric Acid, 4-Nitrophenyl Ester	-	0.19	-	-
58	(S)-(+)-5-Methyl-1-Heptanol	-	0.89	0.44	-
59	Hexadecane	-	1.22	-	-
60	Phthalic Acid, Cyclobutyl Isobutyl Ester	-	0.75	0.74	-
61	Decane, 1-Iodo-	-	1.41	1.20	-
62	Formic Acid, Undecyl Ester	-	0.72	-	-
63	Decane, 2,3,8-Trimethyl-	-	0.30	-	-
64	E,E,Z-1,3,12-Nonadecatriene-5,14-Diol	-	5.64	-	-
65	Oxalic Acid, 6-Ethyloct-3-Yl Isobutyl Ester	-	1.01	-	-
66	Palmitaldehyde, Diallyl Acetal	-	0.36	-	-
67	Tridecanoic Acid, 4,8,12-Trimethyl-, Methyl Ester	-	0.89	0.78	-
68	9-Octadecenamide	-	1.78	2.24	-
69	E-11,13-Tetradecadien-1-Ol Acetate	-	0.45	-	-
70	(Z,Z,Z)-6,9,15-Octadecatrienoic Acid Methyl Ester	-	4.04	-	-
71	Myristic Acid, Glycidyl Ester	-	0.74	-	-
72	(6e)-N-(4-Hydroxy-3-Methoxybenzyl)-8-Methyl-6-Nonenamide	-	7.09	2.25	-
73	Dihydrocapsaicin	-	6.66	1.62	-
74	6-Dimethyl(Trimethylsilyl)Silyloxytetradecane	-	0.38	-	-
75	1,6,10,14,18,22-Tetracosahexaen-3-Ol, 2,6,10,15,19,23-Hexa	-	0.76	-	-
76	Olean-12-En-28-Al, Cyclic 1,2-Ethanediyl Mercaptal	-	0.25	-	-
77	Lanost-8-En-3-Ol, (3.Beta.)-	-	3.80	1.65	-
78	Gamma.-Sitosterol	-	3.67	-	-
79	2-Tert-Butyl-4-(1,1,3,3Tetramethylbutyl)Phenol	-	-	1.21	-
80	Methyl 4-Chlorodecanoate	-	-	0.40	-
81	Phenol, 4-Bromo-2-(1,2-Dimethyl-3-Methylen	-	-	0.81	-

82	Glycine, N-(3-Methyl-1-Oxo-2-Butenyl)-, Methyl ester	-	-	0.46	-
83	Bicyclo[2.2.1]Heptan-2-One, 1,7,7-Trimethyl-3-(2-Nitro-2-Propenyl)-,	-	-	0.60	-
84	Nonane, 5-Methyl-5-Propyl-	-	-	1.36	-
85	Bicyclo[4.1.0]Heptane, 7-Butyl	-	-	0.50	-
86	1,4-Butanediol, 2,3-Diethyl-2,3-Dimethyl	-	-	0.51	-
87	3-Octyne	-	-	0.78	-
88	7,9-Ditert-Butyl-1-Oxaspiro[4.5]Deca-6,9-Diene-2,8-Dione	-	-	0.87	-
89	2-Methyl-6-.Beta.-D-Ribofuranosylimidazo [1,2-C]Pyrimidin-5(6H)-One	-	-	0.63	-
90	2-Dodecanol, 1,1-Dichloro	-	-	0.43	-
91	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester	-	-	9.48	12.51
92	9-Methylnonadecane	-	-	0.98	-
93	(8z)-14-Methyl-8-Hexadecen-1-Ol	-	-	1.86	-
94	Carbonic Acid, Decyl Nonyl Ester	-	-	1.87	-
95	1,2-15,16-Diepoxyhexadecane	-	-	2.31	-
96	Palmitoyl Chloride	-	-	0.42	-
97	Myristic Acid Glycidyl Ester	-	-	4.27	-
98	4-Bromobutanoic Acid, Dodec-3-Ynyl Ester	-	-	0.78	-
99	Linoleyl Acetate	-	-	5.55	5.60
100	Phthalic Acid, Di(6-Methylhept-2-Yl) Ester	-	-	1.90	-
101	4-Cyanobenzoic Acid, Undec-10-Enyl Ester	-	-	0.86	-
102	2,7,8-Trimethyl-2-(4,8,12-Trimethyltridecyl)	-	-	1.22	-
103	11,13-Dimethyl-12-Tetradecen-1-Ol Acetate	-	-	2.50	-
104	β-Sitosterol	-	-	3.80	-
105	Ortho Tert-Butyl Cyclohexyl Acetate	-	-	-	3.09
106	Cyclohexene, 3-Methyl-6-(1-Methylethylidene)	-	-	-	1.96
107	3,4-Dimethoxy Styrene	-	-	-	0.47
108	Cyclohexanol, 2-(1,1-Dimethylethyl)	-	-	-	1.20
109	Phenol, 2-(1,1-Dimethylethyl)-, Acetate	-	-	-	0.50
110	2,5-Diacetylanisole	-	-	-	0.91
111	Acenaphthylene	-	-	-	1.64
112	Methyl (3-Oxo-2-Pentylcyclopentyl)Acetate	-	-	-	3.57
113	1-(4-Isopropylphenyl)-2-Methylpropyl Acetate	-	-	-	5.37
114	(1r,5s,E)-2-Methyl-4-[2,2,3-Trimethyl-6-Methylidenecyclohex-2-En-1-Yl]But-2-Enal	-	-	-	0.81
115	R(-)3,7-Dimethyl-1,6-Octadiene	-	-	-	0.84
116	Decanoic Acid	-	-	-	0.61
117	Tetracosanoic Acid, Methyl Ester	-	-	-	2.52
118	9,12-Octadecadien-1-Ol, (Z,Z)	-	-	-	2.98
119	(2r,5r,6r)-2-T-Butyl-5-Isopropyl-5,6-Dimethyl	-	-	-	0.53
120	Methyl 4,8-Dimethylnonanoate	-	-	-	1.56
121	Tetracosanoic Acid, 3-Oxo-, Methyl Ester	-	-	-	0.95
122	1,2-Benzenedicarboxylic Acid, Dioctyl Ester	-	-	-	1.43

Venn diagram showing the overlap of metabolites of differential stages of seed germination of 'Bullet' chilli detected by GC–MS. Among the identified compounds, five are common to all stages. These compounds

are (1) Tetradecanoic Acid, (2) Hexadecanoic Acid, Methyl Ester, (3) 9-Octadecenoic Acid (Z)-, Methyl Ester, (4) Octadecanoic Acid, Methyl Ester and (5) 9-Octadecenoic Acid (Z)-, Oxiranylmethyl Ester. Venn diagram also revealed that 34 compounds are unique to the dry seed stage, 18 compounds are unique to the imbibed seed stage, 24 compounds are unique to germinating seeds, and 18 specific compounds are present in young seedlings (Table 2). 6 compounds are common to both the dry and imbibed stage, whereas 10 compounds are common to the imbibe and germinating stage. 2 compounds are common to the dry, imbibe and germinating stage, whereas only 1 compound is common in case of dry and germinating stage. Young seedlings showed very few compounds that are/are also present in other stages (Figure 3). Some of the compounds revealed in our study were compared to earlier studies with functional attributes (Table 2). The significance of these compounds was elaborated in the "Discussion" part of this article.

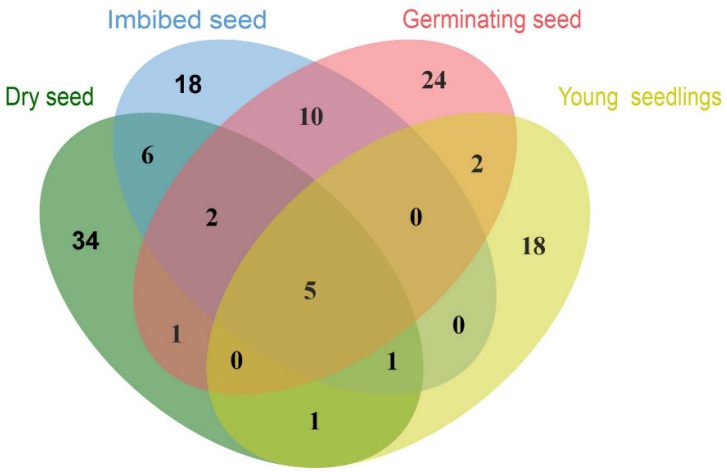

Figure 3. Venn diagram showing the overlap of metabolites obtained from dry, imbibed, germinating seed and young seedlings detected by GC-MS

Table 2. Bioactive compounds showing a significant role in germination in different plants as reported

Compounds	Different stages				Role in seed germination and post-germination growth	References
	D	I	G	S		
β-sitosterol	-	-	+	-	Signaling molecule; Promotes plant embryonic growth, root hair growth	(Gupta, 2020)
Phthalic Acid, Di(6-Methylhept-2-Yl) Ester	-	-	+	-	Allelochemical components and influence growth of neighbouring plants	(Bertin *et al*., 2003; Huang *et al*., 2021)
Benzenedicarboxylic Acid, Dioctyl Ester	-	-	-	+	Check the growth of the fungal pathogen	(Rahman and Anwar, 2006)
Decanoic Acid	-	-	-	+	Inhibit Seed Germination	(Doss *et al*., 1983)
Nonanoic acid, 7-Methyl-, Methyl Ester	+	+	-	-	Inhibit Seed Germination	(Doss *et al*., 1983)

where '**D**' represents Dry Seed, '**I**' represents Imbibed seed, '**G**' represents Germinating seed and '**S**' represents young seedling.

Discussion

Plants possess the ability to produce a diverse array of chemical compounds, which may be categorized into primary and secondary metabolites based on their chemical class, biosynthetic origin, and functional groups. Plants allocate a substantial quantity of secondary metabolites which do not directly contribute to respiratory activities, solute transport, nutrient absorption, photosynthesis and biosynthesis of proteins, carbohydrates or lipids (Saha *et al*., 2020). The production of specific plant metabolites is highly dependent on the species or cultivar, climate and geography, growth phase, harvest time, postharvest factors, and biotic and abiotic factors (Han *et al*., 2021). Successful seed germination is required for the agricultural product to reach its utmost potential. To maximize crop yield, it is necessary to accurately understand the external and internal factors that influence seed germination and subsequent emergence. There are lots of studies about the impact of abiotic factors on seed germination of chilli and allied crops (Sarkar *et al*., 2023). Studies on the internal environment, especially volatiles and metabolic alteration in seeds, are still under the cover of light. However, metabolic profiling is now a valuable tool that offers insights into the phytochemical composition of plants, enabling the identification of a wide range of bioactive molecules and previously undiscovered substances.

Additionally, it gives functional information on the metabolic phenotypes of plants. Exploring phytochemicals offers a valuable avenue for pursuing prospective research and development to enhance the current field of seed sciences. Since volatile chemicals constitute a significant consequence of catabolic events, investigation of these molecules offers an accessible tool to analyze chemical reactions in dry or imbibed or germinating seeds. In the present communication, we have reported the variation of volatile compounds during the course of seed germination and early seedling stage. The GC-MS analysis revealed that each stage has a unique bio-constituent; only five compounds were detected in all extracts: Tetradecanoic Acid; Hexadecanoic Acid, Methyl Ester; Octadecanoic Acid, Methyl Ester; 9-Octadecenoic Acid (Z)-, Oxiranylmethyl Ester; and Octadecanoic Acid, Methyl Ester. In the case of dry seeds, 56 compounds were found, whereas 53 compounds were found in fully imbibed seeds. 13 compounds were found common in these two stages. After germination (5 Days after sowing), when 2 mm radical protrusion takes place, 52 compounds have been found. In the case of germinating seedlings, 28 compounds were detected (Table 1).

A plant or plant organ generates these chemicals to defend itself, but recent research indicates that it is the source of most of these protective, disease-preventing compounds. Some of the components are fatty acids. The structure and function of approximately 400 distinct fatty acids in the plant kingdom are known today (Li *et al*., 2017), and certain of them are required for the appropriate operation of plant cells (Reszczyńska and Hanaka, 2020). Tetradecanoic Acid is a saturated 14-carbon fatty acid found in most animal and vegetable lipids. Due to their strong antiurease, antielastase, and antioxidant capabilities, 3-,6-,7-,9-, and 12-monohydroxy tetradecanoic acid isomers can be employed as innovative biological agents in the agriculture, pharmaceutical, and cosmetic sectors (Sokmen *et al*., 2014). Hexadecanoic acid is thought to be a fatty acid ester that has antioxidant, antimicrobial, flavouring, hypocholesterolemic, and larvicidal properties (Bodoprost and Rosemeyer, 2007; Falodun *et al*., 2009). Acenaphthylene belongs to a class of compounds known as polycyclic aromatic hydrocarbons, which are frequently found in groups of two or more. They can occur in over 100 distinct combinations. However, the most frequent are grouped as 15 (Sahoo *et al*., 2020). However, most ecologists and plant physiologists of the present era have increased their focus on identifying and analyzing various biological parameters concerning the number and quality of secondary metabolites and naturally occurring bioactive chemicals (Karimzadeh *et al*., 2023). To our knowledge, little research has been conducted on the accumulation of fatty acids and other volatiles during the germination of crop plants. Physiological shifting, such as the alteration of dry seed to imbibed seed or germinating seed, can impact the proprietary enzymes involved in the biochemical production of secondary metabolites or alter the associated pathways, diminishing their stability and contribution (Gomez-Maqueo *et al*., 2020; El-Maarouf-Bouteau, 2022; Sarkar and Sadhukhan, 2022). Various alkanes were also revealed in the present study during seed germination and

post-germination events of 'Bullet' chilli. Tetradecane is an alkane with a linear chain, detected in dry seed and imbibed seeds of the cultivar. It functions as a plant metabolite as well as a volatile component and serves as an effective defence induction signal that prepares nearby plants for insect predator attacks (Pan *et al*., 2021). The study found β-sitosterol, a steroidal molecule, in germinating seeds. It exhibits optical activity, and its biological production is facilitated by the deoxyxylulose and mevalonate pathways (Gupta, 2020). This saturated sterol has a distinct role as it promotes plant embryonic growth and is involved in the formation of liquid-ordered (lo) lipid domains (lipid rafts), which are crucial for biological activities such as polarised root hair growth, cytoskeleton reorganization, secondary messenger in signal transduction, cellular sorting, asymmetric distribution of membrane components, and so on (Gupta, 2020). Phthalic acid, Di(6-Methylhept-2-Yl) Ester, is an essential oil found to be present at the seedling stage of the cultivar. This compound is regarded as one of the main allelochemical components of the external environment that impacts the growth of neighbouring plants (Huang *et al*., 2021). Physiological investigations have shown that these chemicals can affect enzyme activity, which could be one of their phytotoxicity mechanisms. Many defensive compounds have been reported in the seedling stage, viz., 1,2-Benzenedicarboxylic Acid and Dioctyl Ester. It was reported that 1,2-Benzenedicarboxylic Acid, Dioctyl Ester, isolated from roots of *Plumbago zeylanica,* effectively checks some fungal species such as *Alternaria alternata*, *Botryodiplodia theobromae*, *Curvularialunata*, *Fusarium equiseti*, *Macrophomina phaseolina* and *Colktotrichum corchori* (Rahman and Anwar, 2006). Some monoterpene molecules also have been found in the case of germinating seeds and young seedlings.

Volatile compounds were considered the regulators of seed dormancy and seed germination. Since seeds contain embryos and nutrient-rich endosperm composed primarily of lipids and carbohydrates, their pre-germination basal metabolism is obligated to emit volatiles that may influence the seed germination process as well as further growth. Some compounds like decanoic and nonanoic acid and their derivatives were reported as germination inhibitors (Doss *et al*., 1983). Several studies have indicated that seeds with lipids as their primary storage component have a shorter lifespan than seeds that primarily store protein or carbohydrates (Nagel and Börner, 2010). However, contrasting findings have been reported, with some studies finding no correlation between seed storage reserves and longevity (Probert *et al*., 2009). The correlation between the amounts of gaseous and volatile metabolites released by germinating seeds and seedlings seems to be generally associated with the quantity of storage compounds found in the seeds.

Seed dormancy, especially physiological dormancy, is influenced by growth-inhibitory and growth-stimulating chemicals in different seed zones (Inácio *et al*., 2013). The seed germination process is intrinsically connected to seed metabolism, which undergoes alterations during its imbibition, radicle emergence, and post-germination growth stages. When a dormant seed absorbs water, the initial low water potential in the dry state leads to a quick absorption of water, which is facilitated by the matrix potential (Weitbrecht *et al*., 2011). The regulation of seed germination, both below and above the optimum, can be influenced by the physiological route that triggers the production of volatile chemicals (Motsa *et al*., 2017). Typically, the life cycle of a seed involves a series of intricate morphological, structural, metabolic, and biochemical transformations. These changes include accumulating various elements, such as proteins and carbohydrates, which need precise monitoring. The integration of germination phenotypes and metabolite profiles yields compelling evidence supporting a correlation between metabolic composition, germination phenotypes, and seed performance (Kazmi *et al*., 2017). During the process of imbibition and germination, seeds undergo metabolic alterations that release volatile compounds. The quantity of these gaseous and volatile metabolites is directly associated with the organic components contained inside the seeds. The more volatile organic compounds may indicate that the studied species accumulate more significant reserves during seed development; consequently, metabolic pathways may be activated effectively after imbibition. The metabolic alterations during germination are of utmost significance, necessitating the documentation and comprehension of the specific modifications. The observed variability in the emission of volatile compounds may be attributed to genetic variations associated with the biochemical makeup of the seeds. Additionally, it is plausible that the variations in volatile

compounds released might be attributed to the chilli cultivars' internal morphological and physiological changes.

Volatiles were not produced by dormant, non-germinating, or dead seeds, demonstrating that metabolic activity inside the seed was necessary for volatile evolution. It is anticipated that there will be discernible changes in metabolite levels that are distinct and time-dependent. The nature and kinetics of these compounds in dry seeds determine how long the seeds survive (Mira *et al*., 2016). Not all volatiles are equally crucial during every stage of germination. Instead, seeds emit different metabolites during different stages. The release of these compounds, particularly in a volatile state, from germinating seeds might potentially impact the germination process of that seed and the direction or further development of the seedling. Many compounds have been reported in dry seeds, but these are absent in imbibed or germinating seeds due to the intactness of the seed coat. The issue of seed coat chemistry has particular significance when considering the existence of chemicals that either impede or stimulate germination or possess hazardous properties (Radchuk and Borisjuk, 2014).

The levels of moisture greatly influence the chemical reactions that take place in seeds. Greater moisture content promotes glycolytic reactions, whereas lower moisture content leads to lipid peroxidation (Mira *et al*., 2010). Several studies have revealed a correlation between seed germination and the emission of volatile chemicals. In their study, (Hailstones and Smith, 1989) discovered a notable association between the germination and vigour of seeds and the relative abundance of volatile aldehydes emitted by soybean seeds that underwent a heating process at a temperature of 130 °C for 2 hours. In a study on soybeans, it was found that germination boosted the levels of many beneficial compounds, including isoflavone aglycones, B soyasaponins, phytosterols, inositol metabolites, antioxidants, and amino acids (Gu *et al*., 2017).

Conclusions

This study offers a fast, precise, and selective method for detecting different phytoconstituents found in 'Bullet' chilli. We compared the variations in metabolite accumulation between dry, imbibed, germinating seeds and young seedlings. The findings can contribute to investigating metabolic changes that occur throughout the germination process over time. A thorough understanding of seed germination physiology is essential for successful crop cultivation. GC-MS studies of germinating seeds of agriculturally significant crops at different phases have facilitated comprehension of how seed contributes to seed germination and post-germination events. Together with the research presented here, these findings suggest that the gradual emission of gaseous and volatile organics may influence the seed germination and post-germination growth of *C. annuum* L. cv. 'Bullet'. It was intriguing to observe that some compounds were lost during imbibition or germination, and others came about. Several commonalities were seen in the volatiles emitted by seeds at various stages, and the pattern and overall composition showed notable variations throughout the stages. This emphasizes the necessity of conducting a broader inquiry to ascertain the impact of seed storage reserve composition of the cultivar and other fundamental characteristics on the generation of seed volatiles.

Authors' Contributions

Conceptualization: AKS and SS; Data curation: AKS and SO; Formal analysis AKS, SG and KB; Investigation AKS, SG and KB; Supervision: SS; Writing - original draft AKS, SG, KB, SO; Writing - review and editing: AKS and SS. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Bardou P, Mariette J, Escudié F, Djemiel C, Klopp C (2014). Jvenn: an interactive Venn diagram viewer. BMC Bioinformatics 15(1):1-7. *https://doi.org/10.1186/1471-2105-15-293*

Bodoprost J, Rosemeyer H (2007). Analysis of Phenacylester derivatives of fatty acids from human skin surface sebum by reversed-phase HPLC: Chromatographic mobility as a function of physico-chemical properties. International Journal of Molecular Sciences 8(11):1111-1124. *https://doi.org/10.3390/i8111111*

Chinnasamy G, Sundareswaran S, Subramaniyan K, Raja K, Renganayaki P, Marimuthu S (2022). Volatile organic compound analysis as advanced technology to detect seed quality in groundnut. Journal of Applied and Natural Science 14(3):885-894. *https://doi.org/10.31018/jans.v14i3.3617*

Doss RP, Kimura Y, Christian JK (1983). Endogenous levels of abscisic acid and decanoic acid in Dutch iris bulbs and the influence of abscisic acid and decanoic acid on iris meristems cultured *in vitro*. Plant Physiology 72(3):713-716. *https://doi.org/10.1104/pp.72.3.713*

El-Maarouf-Bouteau H (2022). The seed and the metabolism regulation. Biology (Basel) 11(2):168. *https://doi.org/10.3390/biology11020168*

Falodun A, Siraj R, Choudhary MI (2009). GC-MS analysis of insecticidal leaf essential oil of *Pyrenacantha staudtii* Hutch and Dalz (Icacinaceae). Tropical Journal of Pharmaceutical Research 8(2):139-143. *https://doi.org/10.4314/tjpr.v8i2.44522*

Gomez-Maqueo X, Soriano D, Velázquez-Rosas N Jiménez-Durán K, Garciadiego MdM, Gamboa-de Buen A (2020). The seed water content as a time-independent physiological trait during germination in wild tree species such as *Ceiba aesculifolia*. Scientific Reports 10:10429 *https://doi.org/10.1038/s41598-020-66759-3*

Gu E-J, Kim DW, Jang G-J, Song SH, Lee J-I, Lee SB, . . . Kim H-J (2017). Mass-based metabolomic analysis of soybean sprouts during germination. Food Chemistry 217:311-319. *https://doi.org/10.1016/j.foodchem.2016.08.113*

Gupta E (2020). β-Sitosterol: Predominant phytosterol of therapeutic potential. In: Mishra P, Mishra RR, Adetunji CO (Eds). Innovations in Food Technology: Current Perspectives and Future Goals. Springer, pp 465-477. *https://doi.org/10.1007/978-981-15-6121-4_32*

Hailstones M, Smith M (1989). Thermally-derived volatile aldehydes in relation to seed viability in soybean seeds. Seed Science and Technology 17(3):649-658.

Han B, Fernandez V, Pritchard HW, Colville L (2021). Gaseous environment modulates volatile emission and viability loss during seed artificial ageing. Planta 253:1-16. *https://doi.org/10.1007/s00425-021-03620-5*

Huang C-M, Chang C-H, Yang C-S, Lin C-L, Chang T-H, Chen C-L, . . . Chen J-J (2021). A new acenaphthylene and bioactive constituents of *Salvia arisanensis*. Chemistry of Natural Compounds 57:991-995. https://doi.org/10.1007/s10600-021-03535-3

Hung R, Lee S, Rodriguez-Saona C, Bennett JW (2014). Common gas phase molecules from fungi affect seed germination and plant health in *Arabidopsis thaliana*. AMB Express 4(1):1-7. https://doi.org/10.1186/s13568-014-0053-8

Inácio MC, Moraes RM, Mendonça PC, Morel LJ, França SC, Bertoni BW, Pereira AM (2013). Phenolic compounds influence seed dormancy of *Palicourea rigida* H.B.K. (Rubiaceae), a medicinal plant of the Brazilian Savannah. American Journal of Plant Sciences 4(1):129-133. https://doi.org/10.4236/ajps.2013.41017

Karimzadeh H, Farhang H, Rahimmalek M, Esfahani MT (2023). Spatio-temporal variations of extract produced and fatty acid compounds identified of *Gundelia tournefortii* L. seeds in central Zagros, Iran. Scientific Reports 13(1):7665. https://doi.org/10.1038/s41598-023-34538-5

Kazmi RH, Willems LA, Joosen RV, Khan N, Ligterink W, Hilhorst HW (2017). Metabolomic analysis of tomato seed germination. Metabolomics 13:1-17. https://doi.org/10.1007/s11306-017-1284-x

Khakimov B, Rasmussen MA, Kannangara RM, Jespersen BM, Munck L, Engelsen SB (2017). From metabolome to phenotype: GC-MS metabolomics of developing mutant barley seeds reveals effects of growth, temperature and genotype. Scientific Reports 7(1):8195. https://doi.org/10.1038/s41598-017-08129-0

Krishnaveni M, Dhanalakshmi R, Nandhini N (2014). GC-MS analysis of phytochemicals, fatty acid profile, antimicrobial activity of *Gossypium* seeds. International Journal of Pharmaceutical Sciences Review and Research 27(1):273-276.

Li C, Cheng X, Jia Q, Song H, Liu X, Wang K, . . . Zhang M (2017). Investigation of plant species with identified seed oil fatty acids in Chinese literature and analysis of five unsurveyed Chinese endemic species. Frontiers in Plant Science 8:224. https://doi.org/10.3389/fpls.2017.00224

Mihaylova D, Popova A, Vrancheva R, Dincheva I (2022). HS-SPME-GC–MS volatile profile characterization of peach (*Prunus persica* L. Batsch) varieties grown in the Eastern Balkan Peninsula. Plants 11(2):166. https://doi.org/10.3390/plants11020166

Mira S, González-Benito ME, Hill LM, Walters C (2010). Characterization of volatile production during storage of lettuce (*Lactuca sativa*) seed. Journal of Experimental Botany 61(14):3915-3924. https://doi.org/10.1093/jxb/erq202

Mira S, Hill LM, González-Benito ME, Ibáñez MA, Walters C (2016). Volatile emission in dry seeds as a way to probe chemical reactions during initial asymptomatic deterioration. Journal of Experimental Botany 67(6):1783-1793. https://doi.org/10.1093/jxb/erv568

Motsa M, Slabbert M, Bester C, Mokwena L, Taylor M (2017). Volatile organic compounds from germinating seeds of *Cyclopia* species as affected by temperature. Seed Science and Technology 45(1):43-55. https://doi.org/10.15258/sst.2017.45.1.22

Nagel M, Börner A (2010). The longevity of crop seeds stored under ambient conditions. Seed Science Research 20(1):1-12. https://doi.org/10.1017/S0960258509990213

Nonogaki H, Bassel GW, Bewley JD (2010). Germination—Still a mystery. Plant Science 179(6):574-581. https://doi.org/10.1016/j.plantsci.2010.02.010

Palekar S, Patel B, Girish N, Menon S (2020). Rapid GC-MS based Phytochemical profiling of extracts of germinating seeds of *Dolichos lablab* Linn. Journal of Plant Science Research 36(1/2):77-84. http://dx.doi.org/10.32381/JPSR.2020.36.1-2.10

Pan Y, Wang Z, Zhao SW, Wang X, Li YS, Liu JN, . . . Xi JH (2021). The herbivore-induced plant volatile tetradecane enhances plant resistance to *Holotrichia parallela* larvae in maize roots. Pest Management Science 78(2):550-560. https://doi.org/10.1002/ps.6660

Probert RJ, Daws MI, Hay FR (2009). Ecological correlates of ex situ seed longevity: a comparative study on 195 species. Annals of Botany 104(1):57-69. https://doi.org/10.1093/aob/mcp082

Radchuk V, Borisjuk L (2014). Physical, metabolic and developmental functions of the seed coat. Frontiers in Plant Science 5:510. https://doi.org/10.3389/fpls.2014.00510

Rahman M, Anwar M (2006). Fungitoxic and cytotoxic activity of a novel compound 1, 2-benzenedicarboxylic acid, diisooctyl ester of *Plumbago zeylanica* linn. Asian Journal of Microbiology Biotechnology and Environmental Sciences 8(3):461-464.

Rao M, Anilkumar C (2020). Conventional and contemporary approaches to enhance efficiency in breeding chilli/hot pepper. In: Gosal S, Wani S (Eds). Accelerated Plant Breeding. Springer. *https://doi.org/10.1007/978-3-030-47298-6_9*

Reszczyńska E, Hanaka A (2020). Lipids composition in plant membranes. Cell Biochemistry and Biophysics 78(4):401-414. *https://doi.org/10.1007/s12013-020-00947-w*

Rohloff J (2015). Analysis of phenolic and cyclic compounds in plants using derivatization techniques in combination with GC-MS-based metabolite profiling. Molecules 20(2):3431-3462. *https://doi.org/10.3390/molecules20023431*

Saha K, Proma RZ, Khan N (2020). Phytochemical screening of plant extracts and GC-MS analysis of n-hexane extract of the leaves of *Cassia alata* Linn. The Journal of Phytopharmacology 9(5):342-347. *https://doi.org/10.31254/phyto.2020.9509*

Sahoo BM, Ravi Kumar BV, Banik BK, Borah P (2020). Polyaromatic hydrocarbons (PAHs): Structures, synthesis and their biological profile. Current Organic Synthesis 17(8):625-640. *https://doi.org/10.2174/1570179417666200713182441*

Sarkar A, Sadhukhan S (2023). Health benefits of phytochemicals in hot pepper (*Capsicum annuum* L.). In: Malik JA, Goyal MR, Birwal P, Watharkar RB (Eds). Plant-Based Bioactive Compounds and Food Ingredients. Apple Academic Press, pp 207-236.

Sarkar AK, Sadhukhan S (2022). Proteomics—a powerful tool for understanding saline stress response in germinating seed. In: Roy S, Mathur P, Chakraborty AP, Saha SP (Eds). Plant Stress: Challenges and Management in the New Decade. Springer, pp 375-399. *https://doi.org/10.1007/978-3-030-95365-2_24*

Sarkar AK, Oraon S, Mondal S, Sadhukhan S (2023). Effect of salinity on seed germination and seedling growth of Bullet cultivar of chilli (*Capsicum annuum* L.). Brazilian Journal of Botany 1-13. *https://doi.org/10.1007/s40415-023-00894-9*

Sokmen BB, Hasdemir B, Yusufoglu A, Yanardag R (2014). Some monohydroxy tetradecanoic acid isomers as novel urease and elastase inhibitors and as new antioxidants. Applied Biochemistry and Biotechnology 172:1358-1364. *https://doi.org/10.1007/s12010-013-0595-2*

Umarani R, Bhaskaran M, Vanitha C, Tilak M (2020). Fingerprinting of volatile organic compounds for quick assessment of vigour status of seeds. Seed Science Research 30(2):112-121. *https://doi.org/10.1017/S0960258520000252*

Weitbrecht K, Müller K, Leubner-Metzger G (2011). First off the mark: early seed germination. Journal of Experimental Botany 62(10):3289-3309. *https://doi.org/10.1093/jxb/err030*

Zhang M, Liu Y, Torii I, Sasaki H, Esashi Y (1993). Evolution of volatile compounds by seeds during storage periods. Seed Science and Technology 21(2):359-373.

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