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INFLUENCE OF ORGANIC FARMING ON VOLATILE COMPOUNDS IN METHANOLIC FRUIT EXTRACTS OF CHILI (*CAPSICUMANNUUM* L.)

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ABSTRACT

In India nearly,3.56 million hectaresof area is being managed organically (certified) with limited internal inputs to keep fruits and vegetables chemical-free. Thepresent study aims to obtain bioactive volatile compounds profile of chili fruits grown under organic and conventional farming system in the same climatic conditions. Fields practicing organic farming ORG I (since last 2-3 year), ORG II (5-6 years) and ORG III (8-9 years) were selected. fruit extracts obtained from three experimental fields and one conventional field were analyzed through Gas Chromatography and Mass Spectroscopy. The current experimental study on comparative analysis of nutrient content and effect of pesticides on chili fruits has been conducted in the year 2018. Study based on soil quality, temperature condition, amount of organic matter, pesticide spray, and year of practicing as an organic field.GC-MS analysis of conventional chili recorded a high number of compounds contrasting to organically grown chili which recorded a lesser number of compounds.GC-MS analysis of chili fruit samples grown in different agricultural conditions showed various volatile compounds. Comaprative data shows higher compounds in conventionally grown samples while lower quantity in organically grown samples. Nutritional compounds like ascorbic acid (Vitamin C) were recorded 13.22% in organic fruits in comparison to conventional *chili* fruits (4.37%).Capsaicinoidsand fatty acid contents were reported manyfold in organically grown chili in comparison to conventional chili fruits. Reports are in favor to promote more and more organic farming systems for nutritional enhancement in food crops.

Keywords: GC-MS, Organic farming, Volatile compounds

INTRODUCTION

Hazards of using synthetic chemicals as fertilizers and pesticides in conventional farming system are well known all over the world. In spite of increasing yield to manifold, green revolution has proved to give uncountable problems to agriculture few to name are water logging, salinity, alkalinity, soil erosion, and resurgence of pests, loss of biodiversity. High doses of fertilizers and pesticides used during Green Revolution has brought significant land and water problems relating to soil degradation over exploitation of ground water and soil pollution **(Singh, 2000)**. Organic farming is a crop production method which maximizes the use of internal farm resources and minimizes use of external chemical fertilizers and pesticides, till the complete self-sufficiency is achieved. Organic agriculture combines tradition, innovations and science to aim sustainable agriculture. It helps to reduce debts through lowering input costs **(Panneerselvam *et al.*, 2010)**.The organic agriculture system, accepted by the European Union and the FAO (Food and Agriculture Organization) as an alternative system to conventional agriculture, appears to be an environmentally friendly growing system. This production method aims to minimize negative effects on the environment and maintains the biological diversity of the soil **(Maeder *et al.*, 2002)**.As per the available statistics, India's rank 8th in terms of World's Organic Agricultural land and 1st in terms of total number of producers as per 2020 data **(FIBL and IFOAM, 2020)**.As on 31st March 2020 total area under organic certification process (registered under National Programme for Organic Production) is **3.67 million Hectare** (2019-20).This includes 2.299 million ha cultivable area and another 1.37 million Hectare for wild harvest collection. According to **APEDA (2018)** total area under organic certification process in India (registered under National Programme for Organic Production) is 3.56 million Hectare (2017-18).India produced around **2.75 million MT** (2019-20) of certified organic products which includes all varieties of food products namely oil seeds, sugar cane, cereals andmillets, cotton, pulses, aromatic andmedicinal plants, tea, coffee, fruits, spices, dry fruits, vegetables, processed foods etc. The production is not limited to the edible sector but also produces organic cotton fiber, functional food products etc.

The 'conventional farming system' favors to imply various external and synthetic chemicals during pre and post harvesting practices. Organic systems had 32% to 84% greater microbial biomass carbon, microbial biomass nitrogen, total phospholipid fatty-acids, and dehydrogenase, urease and protease activities than conventional systems **(Lori *et al.*, 2017)**. The frequency of occurrence of pesticide residues was found to be four times higher in conventional crops, which also contained significantly higher concentrations of the toxic metal Cd **(Baranski *et al.*, 2014)**.

Capsicum annuum L. (family Solanaceae) is used as food ingredient and/or spices in many cuisines attributing their sensory, coloring, flavoring, heat, pungency and unique aroma. Chili is grown all over country as an important vegetable and spice crop. Chili is source of non-climacteric fruits containing various nutrients and vitamins and enriching human diet with color, aroma and pungency. It is well established fact that fruits of chili show antibacterial activity due to phenolics, carotenoids, vitamin C etc. **(Koffi-Nevry*et al.*, 2012)**, antioxidant and anti-carcinogenic activity **(Liao *et al.*, 2020)**. The bioactive compounds of chili fruits available for human consumption showing aforesaid activities provide prevention against various diseases like cardiovascular, obesity, neurological disorder, arthritis etc.

Pepper fruits contain various bioactive compounds attributing to its pungency, taste and aroma like capsaicinoids, vitamin C, decoic acids, esters, etc. Capsaicinoid is a family of compounds that is responsible for giving chili pepper the characteristic pungent taste. Capsaicinoids contain components capsaicin, dihydrocapsaicin, norhydro-capsaicin, norcapsaicin, nornorcapsaicin and nonivamide **(Levono and Prasad, 2017)**. The two major capsaicinoids, capsaicin and dihydrocapsaicin, are responsible for up to 90% of the total pungency of pepper fruits **(Madhumathy*et al.*, 2007)**. Biosynthesis of capsaicin is taxonomically restricted to genus *Capsicum* present in fruit pericarp and seeds. Capsaicinoid compounds have been widely studied and are currently used in the food industry for medical purposes as well as pharmaceuticals and in defensive sprays. Capsaicin is the active principle which accounts most of the pharmaceutical properties of chili. It has been used as an analgesic against arthritis pain and inflammation **(Deal *et al.*, 1991; Hernández-Ortega *et al.*, 2012)**. Concentration of capsaicinoids (group of alkaloids) in the chili fruits depends on genotype, fruit maturity, and conditions of cultivation **(Zewdie and Bosland 2000)**, sometimes it depends on growing and drying conditions **(Wesoowska *et al.*, 2011)**.

Gas chromatography/mass-spectrometry is one of the best tools to analyse essential oils in spices. Bioassays on analyses of the functional components of foods (nutraceuticals) of organic and conventional produce are needed to conclude that organic fruits and vegetables are healthier and safer **(Lotter, 2003)**. To establish the fact that organically grown fruits are nutritionally better over conventionally grown chili, gas chromatography and mass spectroscopy was done for various samples. No proper analysis of effect of agricultural practices on nutritive and pesticides level in chili was carried out. The objective of this research was to analyzeeffect of conversion of chemical farming to organic farming and its impact on bioactive compounds in produce.

MATERIAL AND METHODS

Field area

The study area was located in Jhunjhunu district of Rajasthan (28.1317° N, 75.4022° E) which is 319 m above the sea level. Total 16 farms were selected for study. Four farms practicing conventional farming and 12 farms practicing organic methods of farming were approached to grow chili at the same time. The farms converted from conventional to organic farming approach take time to modify in soil content and environmental conditions. Three types of agriculture farms for organic farming were shown significant difference in terms of soil fertility and its retention capacity**(Choudhary et al., 2020)**. Seeds of chili variety were distributed to farmers of all the farms to grow. Farms were surveyed time to time for proper monitoring on farming practices. Fruits in the month of July were harvested and used for further analyses.

The fruit samples named ORG I (harvested from farms practicing organic farming from 2-3 years), ORG II (harvested from farms practicing organic farming from 5-6 years), ORG III (harvested from farms practicing organic farming from 7-8 years)& CON (harvested from conventional farms) were harvested in July, 2018, dried at room temperature in shadow. The whole fruits including the fruit wall seeds were grinded in mixer to make powder.

Soxhlet Extraction

The extraction for analyzing bioactive constituents was performed using organic solvent extraction method. Soxhlet extraction method was performed by taking 8gm of dried fruit powder. Methanol was selected as extraction solvent due to its high extraction efficiency as compared to other suitable solvents like acetone **(Attuquayefio and Buckle, 1987; Collins et al. 1995)**. Fruit powder was placed into a muslin cloth thimble then extracted with 80% methanol at boiling point (64.7° to 80°) 70° at atmospheric pressure for almost 24 hours. The extraction procedure was carried out till the solvent in siphon tube of Soxhlet apparatus became colorless. The extract was filtered by syringe filters (MCE membrane, pore size 0.45 µ meters). 5ml fruit extract was used for GC-MS analysis.

GC-MS Analytical Conditions

GC-MS analysis was carried out on a Shimadzu GCMSQP2010 Ultra system. The temperature of injector was adjusted to 280°C.The samples were injected in the split mode with split ratio 1/60. 1 ìL injection volume was used. A capillary column Rtx-5MS (5% Diphenyl-95% Dimethyl Polysiloxane), 30 m x 0.25mm x 0.25ìm, was used in the conditions. Inert gas Helium was used as career gas with a constant flow of 1.00 mL/min. Initial temperature of oven was adjusted to 60°C, held for 2 min, increased to 10°C/min up to 260°C and held for 10 min. The MS ionization potential was 70 eV, and Mass scan range was 40-550. Charomatographs were analysed and identified using mass spectroscopy results on NIST database. Four samples were analysed for GCMS and standard deviation from mean was calculated by statistical methods.

RESULTS

The dried powder of pepper fruits were extracted with methanol in Soxhlet apparatus, and analysed for bioactive components in GC-MS. Comparative table showing various compounds are presented in Table 1. The GC-MS analysis revealed the presence of 52 compounds in extract of conventionally grown chili while significantly less i.e. 36, 40 and 39 compounds in ORG I, ORG II and ORG III samples respectively. As reported by the analysis the major components peak area in organic I, II, III and conventionally grown chili were of capsaicin and ascorbic acid (Vitamin C). Ascorbic acid peak area (13.16%, 13.58%, 13.22%) in ORG I, II, and III and very low in conventionally grown chili (4.37%) was recorded (Fig. 1). The results suggest that organically grown chili were three fold higher in vitamin C contents in comparison to conventionally grown fruits of chili. Vitamin E was recorded in ORG III samples in traceable amounts and absent in all others.

Capsaicinoids were recorded significantly high in organic samples. In ORG I Capsaicin, dihydrocapsaicin and Nonivamide were recorded (16.60%, 8.16%, and 0.78% mean quantity peak area. ORG II showed slight decrease in capsaicinoids while ORG II samples gained the lost vigour and showed capsaicin 13.98% and dihydrocapsaicin at 10.64%. Conventional samples were not able to retain capsaicin content as much as ORG samples (10.64 % and 7.04%).

Dichloroacetic acid, tridec-2-ynyl ester was found absent in organic I, II, and conventionally grown chili. It was only present in very high concentration in organic chili (ORG III) with peak area of 23.98%. Desulphosinigrin (absent in most of organic samples) was recorded with very high peak area of 10.15 % in conventionally grown samples.Hexadecanoic acid, methyl ester (1.21%,1.90%,1.54% in organic samples and absent in conventional chili), Hexadeconoic acid (3.96%,4.76%,5.75% and absent in conventional chili), tetradecanoic acid (0.78%, 0.71%, 0.64% and reported to be totally absent in conventional chili) were significantly present in organic fruit samples(table1).

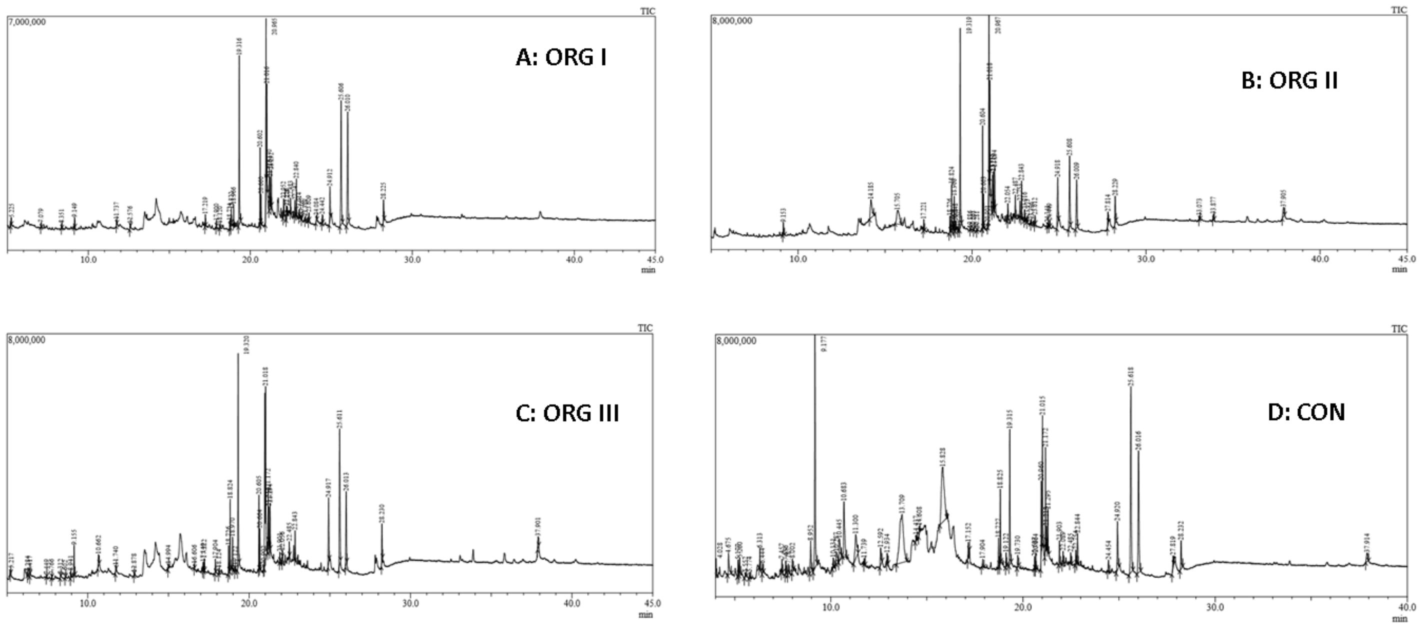

Figure 1 Representative gas chromatography/mass spectrometry chromatograms (full scan mode) of the volatile organic compounds (A): Organic-I, (B): Organic-II, (C): Organic –III and (D)" Conventional Chilli.

Table 1 Volatile organic bioactive compounds generated from chili powder grown under organic and conventional farming systems using Gas Chromatography/Mass Spectroscopy (GC-MS)

Name of Compound[a]	R.Time[b]	Peak Area[c]			
		ORG I	ORG II	ORG III	CON
dl-Glyceraldehyde dimer	4.675	-	-	-	1.21±0.023
1,2-Cyclopentanedione	5.260	0.58±0.014	-	0.19±0.014	0.61±0.021
1-Pentanol, 2-methyl-, acetate	8.952	-	-	-	1.63±0.024
4H-Pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6	9.177	0.68±0.017	0.94±0.017	1.88±0.037	11.82±0.027
5-Hydroxymethylfurfural	10.445	-	-	-	1.73±0.028
Diethoxymethyl acetate	10.662	-	-	1.16±0.027	-
1,2,3-Propanetriol, 1-acetate	10.683	-	-	-	3.87±0.032
N'-(Diaminomethyllidene)butanehydrazide	11.300	-	-	-	2.86±0.023
1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	13.709	-	-	-	12.44±0.023
D-Allose	14.185	-	4.82±0.027	-	-
Desulphosinigrin	15.828	-	5.79±0.033	-	10.15±0.025
Hydroquinone, acetate	17.152	-	-	-	0.37±0.031
Pentadecanoic acid	17.904	0.33±0.024	1.48±0.031	0.80±0.24	0.20±0.027
3,7,11, Trimethyl-8, 10-dodecedienylacetate	18.727	0.44±0.023	1.10±0.026	1.63±0.038	0.81±0.021
3,7,11, Trimethyl-8, 10-dodecedienylacetate	18.825	1.07±0.017	2.95±0.034	3.98±0.048	2.16±0.028
1-(+)-Ascorbic acid 2, 6-dihexadecanoate	19.315	13.16±0.068	13.58±0.172	13.22±0.031	4.37±0.037
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	20.607	5.03±0.043	5.80±0.024	3.77±0.035	0.31±0.028
8,11,14-Docosatricnoic acid, methyl ester	20.660	1.86±0.032	1.86±0.032	2.03±0.021	-
Linoelaidic acid	20.960	12.13±0.073	11.93±0.029	-	1.76±0.025
7-Tetradecenal, (Z)-	21.015	-	5.84±0.033	-	4.09±0.032
cis-9-Hexadecenal	21.016	5.73±0.039	-	-	-
Dichloroacetic acid, tridec-2-ynyl ester	21.018	-	-	23.98±0.023	-
N-Pentadecylacetamide	21.172	2.28±0.051	1.82±0.030	2.90±0.017	2.46±0.028
Octadecanoic acid	21.219	-	-	1.70±0.026	-
Thunbergol	21.295	0.55±0.033	2.54±0.022	2.63±0.032	1.15±0.021
1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10	22.052	1.30±0.028	-	-	-
(E)-3-Methyl-5-((1R,4aR,8aR)-5,5,8a-trimethyl	22.485	0.73±0.021	1.68±0.034	-	0.44±0.028
Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(1)	22.754	-	2.92±0.018	0.81±0.020	0.53±0.028
4-Hexen-1-ol, 6-(2,6,6-trimethyl-1-cyclohexen	22.775	1.30±0.030	-	1.76±0.028	-
trans-Geranylgeraniol	22.844	2.79±0.034	1.22±0.025	-	1.01±0.021
Naphthalene, decahydro-1,6-dimethyl-4-(1-me	23.016	0.41±0.025	1.87±0.025	-	-
Nonivamide	24.454	0.78±0.035	0.40±0.023	-	0.56±0.036
9,12-Octadecadienoic acid (Z,Z), 3-hydroxy-1-(hydroxymethyl)	24.920	3.96±0.044	4.67±0.044	5.75±0.029	2.37±0.041
Capsaicin	25.618	16.60±0.031	8.16±0.034	13.98±0.048	10.64±0.032
Dihydrocapsaicin	26.016	15.26±0.116	5.88±0.034	7.37±0.040	7.04±0.030
trans,trans-9,12-Octadecadienoic acid, propyl	27.819	-	1.27±0.035	-	0.54±0.029
Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl	28.225	2.40±0.030	2.68±0.018	-	-
Octadecanoic acid, 2,3-dihydroxypropyl ester	28.232	-	-	3.10±0.057	1.34±0.027
Vitamin E	33.877	-	0.60**±0.017**	-	-
.gamma.-Sitosterol	37.914	-	-	1.22±0.027	0.78±0.021

[a] Compounds listed in order of elution from SE30 MS column.
[b] Retention time (as min).
[c] Relative area percentage (peak area relative to total peak area %).

DISCUSSION

Various compounds attributing medicinal properties were recorded higher in organically grown chili in comparison to conventional produce. The medicinal uses of chili fruits have been endorsed to the presence of a large number of bioactive compound adapted as free radical scavengers which eventually boosts various cellular defense mechanisms **(Ertani *et al*., 2014; Srinivasan, 2016)**. Role of ascorbic acid as an antioxidant agent and its effects on cancer, blood pressure, immunity, drug metabolism and urinary excretion of hydroxyproline conditions have been discussed earlier **(Silencio Barrita and Santiago Sanchez, 2013)**.In the present study Capsaicin, dihydrocapsaicin and Nonivamide were recorded high in organically grown chili. Dihydrocapsaicin, one more constituent of capsaicinoids, can prompt autophagy in certain transformed cell lines **(Choi et al. 2010)**. Comparative analysis of cytotoxicity, genotoxicity and antioxidant activity of different Capsicum extracts was reported in non-mammalian model **(Bertao, 2016)**.

In some studies Dichloroacetic acid (DCA) was reported to function as a low cost, low toxicity anti-cancer drug **(Bonnet *et al*., 2007; Madhok *et al*., 2010)** which was recorded in organically grown fruits in higher amount in the present study. Esters of fatty acids octodecadienoic acid, pentadecanoic acid, octadecadienoic acid, hexadecanoic acid,linoelaidic acid, tetradecenal,butenedioic acid etc. were recorded in elevated quantity in organic fruits. Hexadecanoic acid, methyl ester and 9,12- octadecadienoic acid (Z,Z)-, methyl ester have shown antioxidant and anticancer properties, respectively **(Wei et al., 2011)**. 9, 12 octadecadienoic acid show antioxidant, cytotoxic and anti-diabetic activities **(Ma et al. 2018)**. In few studies Desuphosinigrin (DSS) promoted the growth of HCT-116 (colon) and NCI H460 (lung) human cancer cells as determined by the MTT assay **(Weil et al. 2004)**. 9, 12, 15-octadecatrienoic acid (Z, Z, Z) - with the retention time at 20.607 was in higher amounts in all organic samples while it was recorded in minimal amount in conventionally grown chili fruits. The compound hasreportedly antimicrobial, anticancer, antipyretic, analgesic, hypo-cholestrolemic and antiseptic activity**(Kalaivani et al., 2012)**.Study by **Shivakummar et al. (2011)**showed that tetradecanoic acid has significant larvicidal and repellent activity against *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes.Various octa and tetradecanoic acids were recorded high in organic samples providing the produce insecticidal property. The volatile compounds present in higher amounts in organic chili contains more vitamins, fatty acids, other important compounds like tetradecanoic proves elevated defense related compounds' synthesis in organic chili. GC-MS studies revealed that chili produced in organic farming systems for more than 5 years are safe and nutritionally superior.

CONCLUSION

GC-MS studies revealed that chili produced in organic farming systems for more than 5 years have advantage to be safe and nutritionally superior over conventionally grown chili.Capsaicin,dihydrocapsaicin,ascorbic acid,linoelaidic acid, and9,12-Octadecadienoic acid (Z,Z) are major bioactive components recorded in higher amount in organically grown chili as compared to conventionally grown chili. Plants start synthesizing defensive bioactive compounds to manage diseases on its own level which is an important step towards sustainable agriculture. Pesticidal residues and its by product are present in conventionally grown chili while products become free from such chemicalseven in traceable amounts. To infer about these compounds further analysis are required**.**Present study strengthen the importance of organic farming practices in vegetable production.

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