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ORIGINAL ARTICLE

Secondary Metabolites and Pesticide Screening in Organic and Inorganic Tomatoes by Gas Chromatography–Mass Spectrometry and Liquid Chromatography/Tandem Mass Spectrometry

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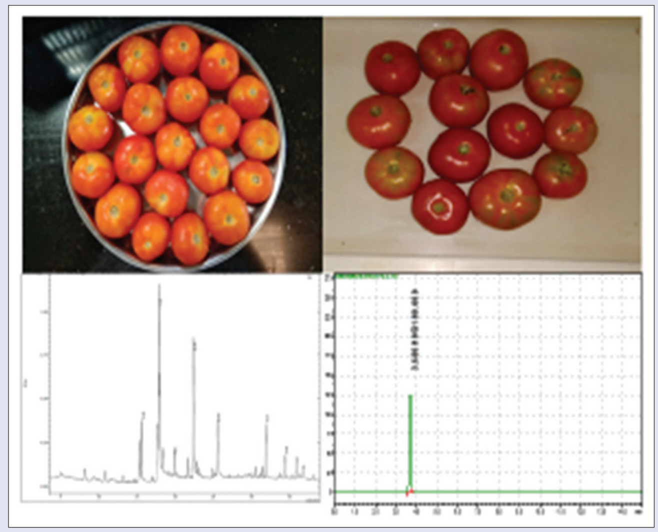
ABSTRACT

Background: Tomato is considered as "protective foods" because of its special nutritive values which are very low in fat and have zero cholesterol level, with an excellent source of lycopene, carotene, anthocyanin, antioxidants, dietary fiber, minerals, and vitamins. **Objective:** The objective of the study was to investigate the occurrence of pesticides and to analyze the secondary metabolite differences in selected organic and inorganic tomatoes (Variety: Sivam). **Materials and Methods:** The samples were analyzed for nearly 167 pesticides using gas chromatography–mass spectrometry and liquid chromatography/tandem mass spectrometry. **Results:** The samples were analyzed for nearly 167 pesticides in which pesticide residues were found to be absent in organic tomatoes; however, inorganic samples indicated the presence of aldoxycarb (aldicarb sulfone) and pymetrozine. The organic tomatoes contain nearly 27 phytocomponents out of which 9,12-octadecadienoic acid (Z, Z)-, methyl ester (15.68%), 9,12,15-octadecatrienoic acid, (Z, Z, Z)- (12.78%), 9,12-octadecadienoic acid (Z, Z)- (10.26%), ethyl oleate (9.48%), linoleic acid ethyl ester (8.09%), oleic acid (7.60%), pentadecanoic acid, ethyl ester (5.08%), n-hexadecanoic acid, and dl-α-tocopherol (4.90%) are major groups, whereas 24 components were noticed in inorganic samples where 9,12,15-octadecatrienoic acid, (Z, Z, Z)- (23.77%), 9,12-octadecadienoic acid (Z, Z)- (11.27%), n-propyl 9,12-octadecadienoate (10.93%), ethyl oleate (8.43%), n-hexadecanoic acid (7.69%), linoleic acid ethyl ester (7.52%), hexadecanoic acid, ethyl ester (4.06%), and dl-α-tocopherol (3.78%) were majorly found. **Conclusion:** Organic tomatoes selected were found to be free from pesticide residues with majority of phytocompounds at higher peaks which are found to be beneficial to fight against many diseases.

Key words: Gas chromatography–mass spectrometry, inorganic, liquid chromatography/tandem mass spectrometry, organic, pesticides, phytocomponents, tomatoes

SUMMARY

- Tomatoes are considered as one of the low-calorie vegetables that hold just 18 calories per 100 g. They are also very low in fat content and have zero cholesterol level and disease-fighting mechanism with an excellent source of lycopene, carotene, anthocyanin, antioxidants, dietary fiber, minerals, and vitamins.
- The present work has been performed to establish the various phytocomponents and pesticide screening using gas chromatography–mass spectrometry and liquid chromatography/tandem mass spectrometry, which creates a better platform in understanding and judging differences between organic and inorganic tomatoes.



Abbreviations used: GC-MS: Gas chromatography–mass spectrometry; LC-MS/MS: Liquid chromatography/tandem mass spectrometry.

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INTRODUCTION

The quality of fruits and vegetables is affected by genetic foundation, growing conditions, inputs used, water source, soil condition, and pre- and postharvest operations. Organically cultivated food utilizes inputs and methods that will help in improving human health and also ecological equilibrium of natural systems, since organic production is carried without pesticides, fertilizers, artificial chemicals, and genetically modified organisms. The insight meaning of organic product is not only in the product but also in its production process.[1] Few researches have shown that the demand for organic agriculture is increasing, as they

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are believed to be healthy by consumers. A survey says that majority of consumers for organic products are women between 30 and 50 years, by giving due consideration to health followed by the absence of usage of pesticide, biological value, and biochemical and organoleptic attributes along with environmental concern.[2]

World has reached 43.1 million hectares of organic land in 2013, with 2 million organic producers. The data say that the total area under organic vegetable production has tripled, from 105,000 hectares in 2004 to 305,000 hectares in 2013. Unfortunately, it represents only 0.5% of the total area of vegetables grown in the world. At present, the United States, China, Mexico, and Italy are the countries with the largest organic vegetable area, with >20,000 hectares each.[3] In spite of high growth, organic farming comprises a quite smaller area of the total acreage in the world. Consumer's preference and expectations toward health effects of organically grown foods are the strongest motives making them to purchase or buy the products, and study results on this topic can count on a high societal interest.[4-6] Nearly 43% of consumers believe that organic foods taste better which becomes a major reason for purchasing organic fruits and vegetables (MORI Poll 2001, cited by Heaton),[7] and also, the levels of some phenolic compounds are known to be present in higher amount in organic fruits and vegetables.[8]

Tomato is the second largest vegetable crop of India. It is one of the most important "protective foods" because of its special nutritive values that are consumed in diversified ways. Tomatoes are considered as one of the low-calorie vegetables and also very low in fat contents with zero cholesterol level, along with an excellent source of lycopene, carotene, anthocyanin, antioxidants, dietary fiber, minerals, and vitamins. The antioxidants present in them are scientifically proved to be protective in fighting against cancers, including colon, prostate, breast, endometrial, lung, and pancreatic tumors. In the present work, gas chromatography–mass spectrometry (GC-MS) and liquid chromatography/tandem mass spectrometry (LC-MS/MS), a chromatographic methodology, was used to identify volatile organic compounds (VOCs) and screening of 167 pesticide residues in selected organic and inorganic tomatoes produced in Tamil Nadu, India.

MATERIALS AND METHODS

Fresh samples of organic and inorganic tomato fruits were procured from Coimbatore and Thanjavur, Tamil Nadu, India, and screened for secondary metabolites and pesticides using chromatography–mass spectrometry/mass spectrometry (GC-MS) electron ionization mode and LC-MS/MS (Make: Shimadzu Corporation, Kyoto, Japan, Model: LCMS 8040, triple quadrupole). Every effort was made in maintaining the quality of samples throughout the supply chain.

RESULTS AND DISCUSSION

Gas chromatography–mass spectrometry

GC-MS helps in quantification of VOCs in fruits and vegetables, which poses a continuous analytical challenge. These problems are mainly due to the low levels of VOCs present in some complex matrices along with huge chemical diversities. GC-MS successfully maximizes the extraction and exhaustive identification of VOCs present in the sample analyzed.[9] It exhibits an extraordinary detection level allowing the identification of a large number of VOCs in plant tissues and other biological matrices.[10]

The compounds present in organic and inorganic samples were identified by GC-MS analysis [Figures 1 and 2]. The active principles with their retention time, molecular formula, molecular weight, and concentration (%) are presented in Tables 1 and 2. Nearly 27 compounds were identified in organic and 24 in inorganic tomato samples by GC-MS. The major components present were 9,12-octadecadienoic acid (Z, Z)-, methyl ester (15.68%), 9,12,15-octadecatrienoic acid, (Z, Z, Z)- (12.78%), 9,12-octadecadienoic acid (Z, Z)- (10.26%), ethyl oleate (9.48%), linoleic acid ethyl ester (8.09%), oleic acid (7.60%), pentadecanoic acid, ethyl ester (5.08%), n-hexadecanoic acid, and dl-α-tocopherol (4.90%), and various other compounds were identified as low level in organic samples. Whereas, main compounds found in inorganic samples were 9,12,15-octadecatrienoic acid, (Z, Z, Z)-(23.77%), 9,12-octadecadienoic acid (Z, Z)- (11.27%), n-propyl 9,12-octadecadienoate (10.93%), ethyl oleate (8.43%), n-hexadecanoic acid (7.69%), linoleic acid ethyl ester (7.52%), hexadecanoic acid, ethyl ester (4.06%), and dl-α-tocopherol (3.78%), respectively. These phytochemicals are responsible for various pharmacological actions such as antimicrobial and antioxidant anti-inflammation, anticancer, hepatoprotective, diuretic, antiasthma activities.

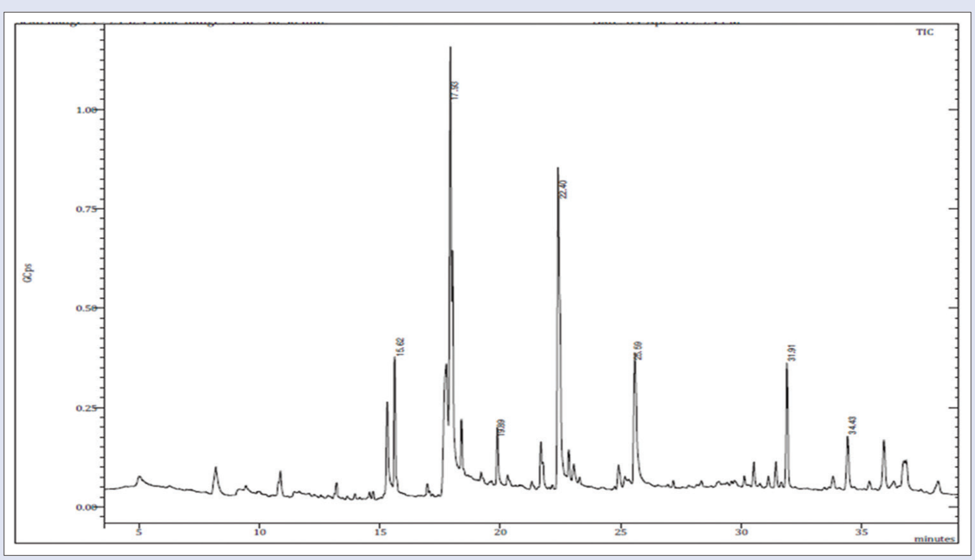

Figure 1: Gas chromatography–mass spectrometry analysis of organic tomatoes

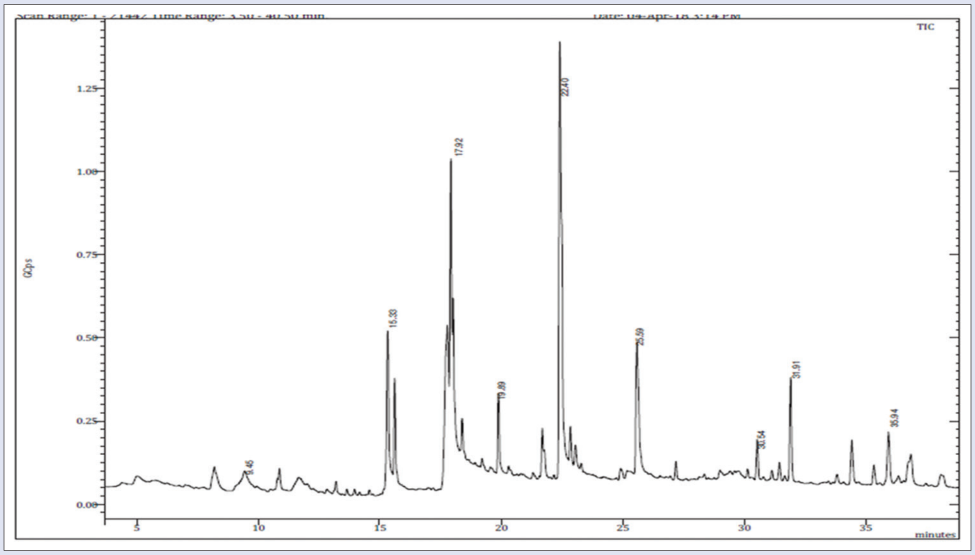

Figure 2: Gas chromatography–mass spectrometry analysis of inorganic tomatoes

Table 1: Phytocomponents identified in organic tomatoes by gas chromatography-mass spectrometry

RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
5.00	d-Mannose	$C_6H_{12}O_6$	180	0.09
8.19	1-Dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	228	2.22
10.88	Decane, 2,3,5,8-tetramethyl-	$C_{14}H_{30}$	198	0.98
15.30	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.90
15.62	Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	270	5.08
16.98	9,12-Octadecadienoylchloride, (Z, Z)-	$C_{18}H_{31}ClO$	298	0.36
17.76	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280	10.26
17.93	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$	294	15.68
18.02	Ethyl oleate	$C_{20}H_{38}O_2$	310	9.48
18.39	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	2.44
19.89	Octadecanedioic acid	$C_{18}H_{34}O_4$	314	2.20
20.31	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z)-	$C_{21}H_{36}O_4$	352	0.53
21.69	8,11,14-Eicosatrienoic acid, (Z, Z, Z)-	$C_{20}H_{34}O_2$	306	1.56
22.40	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	$C_{18}H_{30}O_2$	278	12.78
22.85	Oleic acid	$C_{18}H_{34}O_2$	282	7.60
23.06	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	1.22
23.29	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	312	0.29
24.92	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	318	1.17
25.59	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	8.09
27.18	β-D-Mannofuranoside, farnesyl-	$C_{21}H_{36}O_6$	384	0.23
30.13	Ergosta-5,22-dien-3-ol, acetate, (3β,22E)-	$C_{30}H_{48}O_2$	440	0.34
30.53	6,7-Epoxypregn-4-ene-9,11,18-triol-3,20-dione, 11,18-diacetate	$C_{25}H_{32}O_8$	460	0.90
31.13	Stigmasta-5,22-dien-3-ol, acetate, (3β)-	$C_{31}H_{50}O_2$	454	0.38
31.91	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430	4.90
34.43	Stigmasterol	$C_{29}H_{48}O$	412	2.54
35.93	β-Sitosterol	$C_{29}H_{50}O$	414	2.53
36.75	Lupeol	$C_{30}H_{50}O$	426	1.23

RT: Retention time

Liquid chromatography/tandem mass spectrometry

LC-MS/MS is a more valuable and unique analytical technique to meet the requirements in complex samples. LC-MS/MS with a triple quadrupole in multiple reaction monitoring mode has become the most widely used method for monitoring and quantitation of pesticides in food products.[11-15] The main benefit of tandem mass spectrometry is based on its high sensitivity, reduction of sample treatment steps and reliable quantitation, and confirmation at the low required concentrations.[14]

There are nearly >900 pesticides worldwide, both legally and illegally on food products as well as in the treatment of soil and crops. Each and every individual pesticide has MRLs for both food, water, and soil to protect consumers that need to be monitored for food, especially fruits and vegetables. Green pepper, tomatoes, and oranges were evaluated for screening, quantitation, and confirmation of different pesticides commonly used in agriculture using LC-MS/MS. The results showed that sensitivity and selectivity achieved with this methodology are appropriate for large-scale multiresidue analysis of pesticides in food samples according to the requirements.[16]

Table 2: Phytocomponents identified in inorganic tomatoes by gas chromatography-mass spectrometry

RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
5.00	d-Mannose	$C_6H_{12}O_6$	180	0.02
8.19	β-D-Glucopyranose, 4-O-β-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	1.44
9.45	Undecanoic acid	$C_{11}H_{22}O_2$	186	0.74
10.87	Methoxyacetic acid, 2-tridecyl ester	$C_{16}H_{32}O_3$	272	0.74
11.67	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	0.02
13.21	1-Dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	228	0.29
15.33	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.69
15.63	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	4.06
17.78	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280	11.27
17.92	n-Propyl 9,12-octadecadienoate	$C_{21}H_{38}O_2$	322	10.93
18.02	Ethyl oleate	$C_{20}H_{38}O_2$	310	8.43
18.39	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	2.34
19.89	Octadecanedioic acid	$C_{18}H_{34}O_4$	314	2.55
21.69	8,11,14-Eicosatrienoic acid, (Z, Z, Z)-	$C_{20}H_{34}O_2$	306	1.58
22.40	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	$C_{18}H_{30}O_2$	278	23.77
22.85	Oleic acid	$C_{18}H_{34}O_2$	282	2.53
23.05	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268	1.49
25.59	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	7.52
27.19	Cholestan-3-ol, 2-methylene-, (3β,5α)-	$C_{28}H_{48}O$	400	0.62
30.54	γ-Tocopherol	$C_{28}H_{48}O_2$	416	1.36
31.19	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430	3.78
34.43	Stigmasterol	$C_{29}H_{48}O$	412	1.91
35.94	β-Sitosterol	$C_{29}H_{50}O$	414	2.52
36.78	Lupeol	$C_{30}H_{50}O$	426	2.36

RT: Retention time

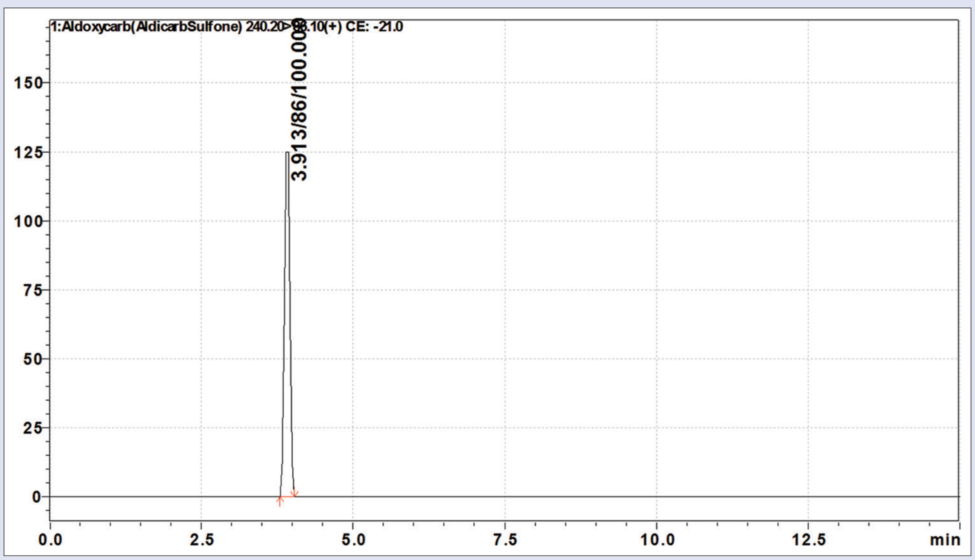

Figure 3: Liquid chromatography/tandem mass spectrometry screening of chromatogram of aldoxycarb pesticide in inorganic tomatoes

The presence and absence of pesticide residues were determined through screening by comparing organic and inorganic tomato samples using LC-MS/MS technique. The pesticides were found to be "absent" in organic samples, whereas inorganic tomatoes showed the presence of two pesticides out of 167 [Figures 3-6], such as aldoxycarb (aldicarb sulfone) and pymetrozine at absolute (36 and 41) and relative intensity (100). Aldicarb is mainly a carbamate insecticide which is the active substance in the pesticide Temik and also effective against aphids, spider mites, thrips, lygus, fleahoppers, and leafminers and commonly used as nematicide.[17] Aldicarb causes headache, nausea, tearing, weakness, blurred vision, sweating, and tremors in humans and also fatal since it can paralyze the respiratory system in humans (US Environmental Protection Agency) if exposed to higher amounts. Pymetrozine is a highly active and specific insecticide against insect pests. It is used worldwide for control of aphids and whiteflies in all field crops, fruits, and vegetables. It is considered as an eco-friendly insecticide. The level of toxicity for mammals is low and safe for most non-target arthropods, birds, fish, and natural enemies for mites with an extraordinary performance in integrated pest management.

CONCLUSION

LC-MS/MS and GC-MS are powerful methods used to determine pesticide residues and secondary metabolites in organic and inorganic tomatoes. The results of monitoring indicate that, among

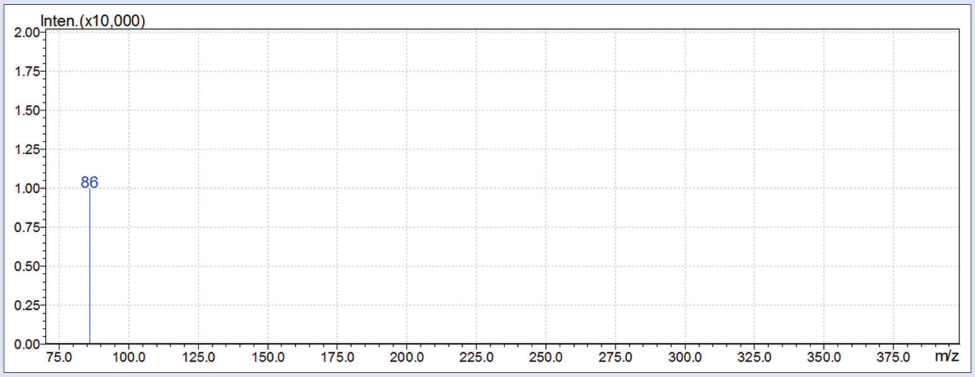

Figure 4: Liquid chromatography/tandem mass spectrometry spectrum of aldoxycarb in inorganic tomato sample

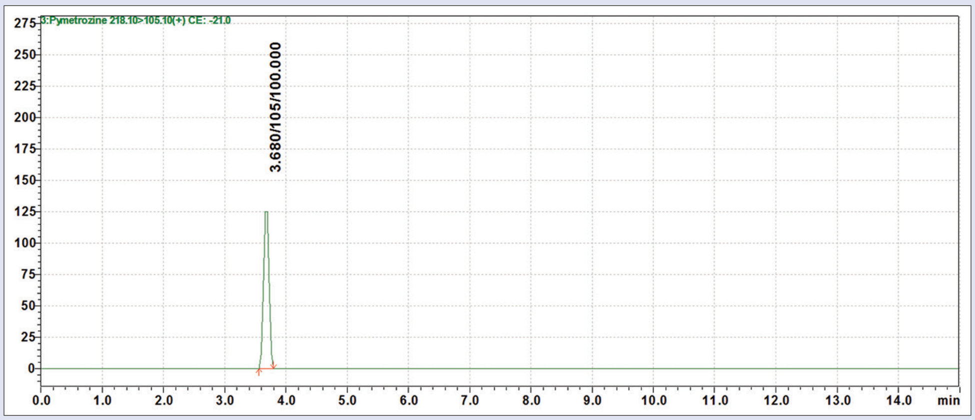

Figure 5: Liquid chromatography/tandem mass spectrometry screening of chromatogram of pymetrozine pesticide in inorganic tomatoes

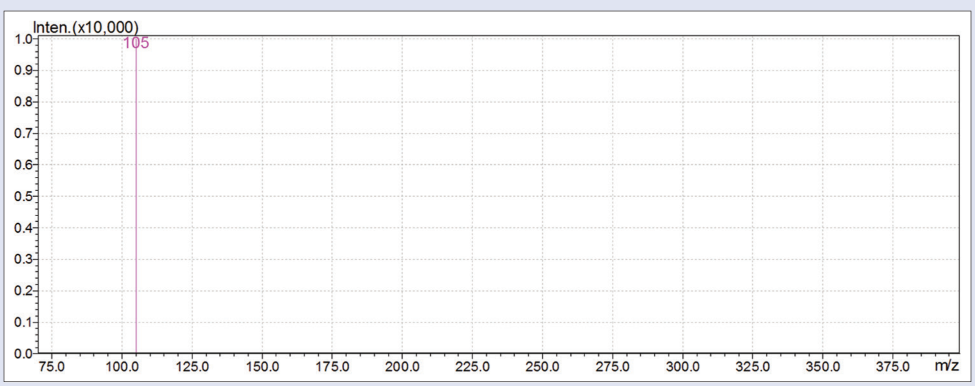

Figure 6: Liquid chromatography/tandem mass spectrometry spectrum of pymetrozine in inorganic tomato sample

167 pesticide screening, inorganic samples contained two residues such as aldoxycarb (aldicarb sulfone) and pymetrozine and organic tomatoes were free from pesticide residues. The presence of phytocomponents and its range was noticeably high in organic compared to inorganic tomatoes. The present work serves the beneficial effects of organic tomatoes in fighting against several diseases successfully, leading to consumer health and ecosystem protection.

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Conflicts of interest

There are no conflicts of interest.

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