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Pesticide And Phytochemical Analysis Of *Amaranthus gangeticus* And *Coriandrum sativum* Plant Samples Of Tirunelveli District- A Report

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ABSTRACT:
Farmers use a variety of pesticides to save the plants from pests and diseases. But , it is proved that the pesticides may be detrimental to human health and ecosystem. Two leafy vegetables – Amaranthus gangeticus and Coriandrum sativum have been chosen for this study and subjected to GCMS and Phytochemical analysis to find out the presence of pesticides and to examine the effect of pesticides on these vegetables. GCMS analysis of Amaranthus gangeticus showed the presence of compounds such as Methyl, Oxirane, Naphthalene, Saline and Hexadiene. The GCMS profile of Coriandrum sativum showed the presence of compounds such as Formamide, Benzenmethanamine, Dichloroacetic acid and Benzaldehyde. Phytochemical analysis proved the showed the presence of tannins and flavonoids in Amaranthus gangeticus and alkaloid in Coriandrum sativum.

Key words: *Amaranthus gangeticus, Coriandrum sativum,* GCMS, Phytochemical analysis .

INTRODUCTION:

Leafy vegetables are plant leaves eaten as a vegetable. Nearly one thousand species of plants with edible leaves are commonly used. Leaves are photosynthetic tissues. Leafy vegetables are low in calories and fat. But they contain high levels of protein, dietary fiber, vitamin C and vitamin K (Nast[1], 2014).

In order to save the vegetables from pests and diseases, farmers use a wide range of pesticides. Pesticides help the farmers in increasing their production. At the same time, they are proved to be detrimental to human health and ecosystem. (Tadesse[2] and Asferachew, 2008).

Pesticide contamination, especially in developing countries is largely due to unsafe use or misuse of pesticides. Use of faulty and improper

maintenance of spraying equipment, lack of use of protective gear and appropriate clothing during handling of pesticides may cause harmful effects (Damalas[3] et al.,2006; Ajayi and Akinnifesi, 2008; Sosan and Akingbohungbe, 2009).

In increasing the agricultural productivity, pesticides play a larger role. But reliance on pesticides will have adverse effects on the environment and human health. Real risk to human health is through exposure to residues in primary and derived agricultural products (Cecchi[4] et al., 2012).

Use of pesticides per hectare of land is on the increase in India. On an average 1.2 gha -1 was the average use of pesticides per hectare in 1953. It has increased to 377 gha- 1 in 1985 and to 431 gha -1 in 1992.

In vegetable production, India enjoys the second position next only to China. Nearly 13 per cent of the world output of vegetables is produced in India. The effect of chemical pesticides is more harmful in vegetables. Scientists have called for the rational use of pesticides and minimizing the problems related to health and environment to get rid of the inappropriate application of pesticides.

For the present study, commonly used leafy vegetables *Amaranthus gangeticus* and *Coriandrum sativum* are selected by the scholar.

In tropical and temperate regions *Amaranth* is grown. *Amaranth* is used as food in countries like Africa, India and Nepal.

The height of the plant varies from 0.3 – 2 m depending on the species, growth habitat and environment. The leaves are green or purple in color with slender stalks. Seeds are small, shiny and black in color.

Insects such as moth larvae, fleas, stinkbugs and blowflies attack *Amaranthus*. Potentially significant insect pest of *Amaranthus* are Tarnished plant bug and *Amaranth* weevil. Various organic insecticides are used for *Amaranthus*.

NUTRITIONAL CONTENT:

Amaranthus gangeticus contains important nutritive values such as energy, moisture, protein, fat carbohydrate, fiber, iron and phosphorus.

Coriander leaves are mainly water, with a good amount of vitamins A, C, and K, as well as minerals like calcium, iron, manganese, and phosphorus.

REVIEW

Review of literature enables the scholar get an in-depth idea of the area of research. It also helps the scholar to carry on her study in a different way from the existing studies and do the research on untouched areas.

Liu et al., (2001) was of the opinion that millions of tons of pesticides are applied in modern farming to increase the productivity and production of crops by controlling the harmful effects caused by insects, fungi, bacteria, viruses etc.

Different non-chemical control methods must be let known to the farmers to adopt integrated pest management practices according to Jeyanti[5] and Kombairaju (2005). Goel and Aggarwal, (2007) point out that acute poisoning with pesticide is a global public health problem that accounts for nearly 300,000 deaths all over the world annually.

Jallow et al., (2007) assessed the level of pesticide residue in commonly consumed fruits and vegetables. Venskutonis[6] and Kraujalis, (2013) reviewed the nutritional content of *Amaranthus* seeds and vegetables. If the present level of harmful pesticide use continues, before things go

out of hand, farmers who use pesticides for living organs including man will become extinct. The consumers who consume them must be educated of consequences of the over use of pesticides.

AIM OF THE STUDY

The primary aim of this study was to characterize the pesticides present in the plants *Amaranthus gangeticus* and *Coriandrum sativum* found in Tirunelveli region, using Gas Chromatography Mass Spectroscopy and phytochemical profiling techniques and to find out the impact of pesticides on primary metabolites.

MATERIALS AND METHODS

In the present study, the selected leafy vegetable, *Amaranthus gangeticus* was subjected to GCMS analysis to find out the effect of pesticides.

Gas Chromatography and Mass Spectroscopy

GCMS analysis was conducted using the JOEL GCMATE 11 with data system double focusing instrument. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV Helium gas as a carrier gas at a constant flow rate of 1 ml/ minute and an injection volume of 2 µl was employed. The injector temperature was maintained at 250^{o}C. The ion-source temperature was 200^{o}C, the oven temperature was maintained from 110^{o}C. With an increase of 10 C/minute to 200^{o}C, then 5 C /minute to 280^{o}C, ending with a 9 minutes isothermal at 280^{o}C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 minutes, and the GCMS running time was 36 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The procedure of Kakimoto[8] et al., (2005) was followed.

Identification of components: Interpretation of mass spectrum was conducted using data base of National Institute of Standard Technology and Wiley spectra Libraries. The molecular weight, molecular formula and the number of hits used to identify the name of the compound from NIST and Wiley spectra Libraries.

PHYTOCHEMICAL EVALUAION

Primary components of plants such as carbohydrates, proteins, nuclei acid, chlorophylls and hemes are found throughout the plant kingdom and are central to the metabolism of plants. Over and above the primary metabolism, there are numerous secondary plant products such as tannins, glycosides, phenols, flavonoids, phenolic compounds and quinones.

TEST FOR TANNINS (Sofowara,1993)

When 1ml of lead acetate solution was added with 2ml of alcoholic extract, white precipitate is formed and this shows the presence of tannins.

TEST FOR GLYCOSIDES (Trease[7] and Evans, 1989)

With 2ml of chloroform 2ml of test solution and few drops of ammonium solution were added. Formation of pink color indicated the presence of glycosides.

TEST FOR PHENOLIC COMPOUNDS (Sadasivam[10] and Manickam,1998)

Along with 2ml of alcoholic extract, a few drops of neutral ferric chloride $FeCI_2$ was added. Intense color is developed and it indicates the presence of phenolic compound.

TEST FOR QUINONES (Evans, 1996)

When the test solution was treated with a few drops of concentrated H_2SO_4, red color was formed and it indicated the presence of quinone compound.

TEST FOR FLAVONODS (Harbone,1973)

With the test solution, few drops of concentrated hydrochloric acid and a few magnesium turnings were added and boiled for five minutes. Orange or red color appears and this indicated the presence of flavonoids.

TEST FOR ALKALOIDS (Evans,1996)

Test solution was treated with few drops of Mayor's reagent. Yellow color is formed and this shows the existence of alkaloids

TEST FOR SAPONINS (Evans, 1989)

Aqueous crude plant extract was mixed with 5ml distilled water.in a test tube and mixed for some time. Then few drops of olive oil were mixed and stirred vigorously. Foam like something appears and it shows the presence of saponins.

TEST FOR COUMARINS (Harborne 1973)

Few drops of moistened extract were taken in a test tube. Test tube's mouth was covered with filter paper treated with 1 N NaOH solution. Appearance of yellow color indicates the presence of coumarins.

RESULTS AND DISCUSSION

It is a known fact that leafy vegetables have low calories and fat. So also, high levels of protein, dietary fiber, vitamin C carotenoids, folate, manganese and vitamin K are present. As leafy vegetables are photosynthetic, they contain a high

level of vitamin K. Phylloquinone is involved in photosynthesis (Kessler and Glaucer, 2014). They also contain amino acids, methionine and polyphenols (Hussain et al., 1999). Consumption of leafy vegetables give several health benefits. Leafy vegetables are mixed with food. This gives healthy body and immune system. It is consumed for enjoying blood purification and strong respiratory system. Alkaline minerals supplied by green vegetables neutralizes the acidic conditions caused by the environment. It also filters out pollutants.

With the launch of Green Revolution, use of chemical pesticides has become inevitable. But the over use of the pesticides affects the environment and affects the human health. With the change in the crop varieties and the introduction of short duration crops and increase in the number of crops in an agricultural year, use of more and more of pesticides and fertilizers have become inevitable. This is also associated with rapid spread of pests and diseases. Several investigations have shown that pesticide contamination is there in several commodities in modern cultivation.

In the present study, two vegetables commonly in use among the general public of the study area (Tirunelveli District) namely *Amaranthus gangeticus* and *Coriandrum sativum* have been selected and Gas Chromatography Mass Spectroscopy analysis was tried to examine the presence of pesticides if any and Phytochemical analysis was tried to find out the effect of pesticides detected. Generally, *Amaranthus gangeticus* is eaten in half cooked stage and *Coriandrum* is used to get flavor in dishes. In order to control the pest to overcome the crop loss, the farmers of this district use pesticides. GCMS analysis tried in the selected two vegetables showed the presence of harmful chemicals and pesticide residues in both the vegetables.

Retention time varying from 4.5 to 14 minutes with about 41 compounds identified, in *Amaranthus gangeticus* 15 peaks have been identified. The peaks observed were 4.791, 5.113, 6.276, 7.051, 7.363, 7.222, 7.959, 8.403, 8.857, 9.812, 10.095,11.448, 11.712 and 13.594 (Table 1a (GCMS).

Table 1a GCMS Profile of *Amaranthus gangeticus*

Plant Name	Retention time	Compounds identified
Amaranthus gangeticus	4.791	2-Octyn-1-ol 1,3,4-Hexatrirne, 1-methoxy 5-Methyl-1-hexyn-3-ol 2-Hexyn-1-ol 5-Hexen-2-ol, 5-methyl-
	5.113	Oxirane, ((dodecyloxy) methyl)- 2-Undecene, 4-methyl-Alloaromadendreme Naphthalene, 1,2,3,4,4,5,6,8-
	5.699	alpha-Bainesene alpha-Guaiene beta-Humulene
	6.276	Cyclopropanecarboxylic acid, 1-amino- Silane, ethyl- 1.5-Hexadiene, 3,4-dimethyl-
	7.051	Acetylene dicarboxamide 2-Methyl-6-methylene-octa-1,7-dien-3-ol 3,3,5,5-Tetramethylcyclopentene
	7.722	2-Hydroxy-1-isoindolinone Phthalic acid, monoethyl ester Phenol, 2-amino-4-(1H-1,2,3,4-tetrazol-1-y
	7.959	9-Borabicyclo[3.3.1]nonane, 9-methyl- 3-Octen-1-yne.(E)- Paromomycin
	8.403	Azulene, 1,2,3,3,4,5,6,7-octahydro- Alloaromadendrene Longifolene
	8.857	Furazancarboxylic acid, 4-amino-
	9.812	Z-10-Pentadecen-1-ol
	9.812	3-Piperidinol, 1,4-dimethyl-, cis- 9-Borabicyclo 3.3.1]nonane, 9-methylthio- 4-Hexenoic acid, 6-hydroxy-4-methyl- 1.3-Bis-(2-cyclopropyl,2-methyl
	10.095	4,6-Dimethyl-3-nitro-2(1H)-pyridinone Cyclohexane-1,3-dione, 2-allylami 2-ethoxy-3,5-hexadiene
	11.448	2- Nonadecanol
	11.712	1-Cyclohexyl-2-propen-1-ol 3-Methoxymethoxy-2,3-dimethylundec
	13.594	[1,2,4 Triazolo[1,5-a]pyrimidine- [1,2,4 Triazolo 1,5-a]pyrimidine-9-Borabicyclo[3.3.1]nonane, 9-[3-

Among the compounds identified, important are 5-Methyl-1-hexyn-3-01 which is a flammable liquid chemical; Oxirane a harmful chemical, Naphthalene and Hexadiene. Over and above these, very toxic compounds, Acetylene dicarboximide and Phthalic acid, components of shampoo, soap etc were also detected. Crude oil or coal tar is the raw material used for Naphthalene making. Naphthalene is there in cigarette smoke, car exhaust, and forest fire smoke. Naphthalene is used as a precursor of other chemicals. Even the blood cells would be damaged if large volume of naphthalene is exposed to human body (Santuci and Shah, 2000). If children ingest mothball or deodorant blocks, they may be affected by reaching a stage of hemolytic. So also, it may cause confusion, nausea, vomiting, diarrhea, blood in the urine and jaundice. Some toxic chemicals are also

identified. Exposure to diethyl phthalate which is a harmful one identified by GCMS test can cause damage to the nervous system and to the reproductive organs of both male and females.

The GCMS test conducted on *Coriandrum sativum* has proved the presence of 48 compounds distributed along 15 peaks at RT 4.536, 5.065, 6.096, 6.370, 6.607, 6.966, 7.949, 8.375, 9.869, 10.493, 11.183, 11.703 and 12.658 (Table 1b: GCMS). This test has shown the presence of a compound, Formamide, a harmful chemical at RT 4.536. So also, some other chemicals such as Benzene methenamine (RT 6.966), dichloroacetic acid allyl ester at RT 6.370 and Benzaldehyde, etc. Experiment with animals show the fact that if Formamide is repeatedly inhaled, ingested or observed through the skin, it can cause embryotoxicity. It is a solvent for many ionic compounds and is used as a solvent for resins and plasticizers (Hohn, 1999). Benzene methenamine causes irritation to eye, skin, and other organs. On the other hand, Dichloroacetic acid allyl ester is a harmful chemical which causes acute toxicity, severe skin burns and eye damage. It may even cause cancer.

Table 1b GCMS Profile of *Coriandrum sativum*

Plant Name	Retention time (min)	Compounds identified
Coriandrum sativum	4.536	Formamide N methyl-N-phenyl Octen -3 yne Adamantane-1-sulfinic acid benzylamide
	5.065	1,3 Dioxane 2 propanol 2 methyl 1,2,3-Triazolo 4,5-f benzotriazole-4, 1,5 Hexadiyne
	6.096	2,3-Hexadiene, 2-methyl- 2 Methylene cyclohexanol Furan, tetrahydro-3-methyl-4-methylene-
	6.37	Dichloroacetic acid, allyl ester2-Azatricyclo[4.3.1.1(4,8)]undecane 2 Hexyn-1-ol
	6.607	1-[.alpha.-(1-Adamantyl) 1,2-Benzenedicarboxylic acid, Spiro[2.2]pentane-1-carboxylic acid,
	6.966	Benzenemethanamine, N,N-dimethyl- 2-Methylenecyclohexanol Ethanol, 1-(methylencyclopropyl)-
	7.949	2H-Benzocyclohepten-2-one, 3,4,4,5,6,7,8,9 1,2,4-Triazolo[4,3-a]pyridin-3(2H)-one, 5- 1-(4-Amino-furazan-3-yl)-5-methyl-1H-
	8.375	Cyclobutanone, 2-methyl-2-oxiranyl- 1,3-Bis-(2-cyclopropyl, 2-methylcyclopropyl)- 7-Oxabicyclo[4.1.0]heptane, 3-oxiranyl-
	8.857	6-Oxabicyclo[3.2.1]oct-2-ene-8-carboxylic acid, 1,8-dimethyl-7- oxo-, anti-(.+/-.)- Spiro {6,6-dimethyl-2,3-diazobicyclo [3.1.0] Carbonic acid, tetradecyl 2,2,2- n-Heptadecanol-
	9.632	Bromoacetic acid, tetradecyl ester
	9.869	Benzaldehyde, 2-nitro-, diamino Bicyclo[10.1.0]trideca-4,8-diene-13-2,4-Dimethyl-3-nitrobicyclo[3.2.1]octan-8-one
	10.493	cis-9,10-Epoxyoctadecan-1-ol 1,13-Tetradecadiene 8-Dodecen-1-ol, (Z)-
	11.183	3-Bromo-7-methyl-1-adamantanecarboxylic 1-(2-Adamantylidene)semicarbazide Tricyclo[6.3.3.0]tetradec-4-ene
	11.703	Butanoic acid, heptafluoro-, 4-butoxy-4- Cyclopentadecanone, 2-hydroxy- Cyclobutanone, 2-(1,1-dimethylethyl)-
	12.658	9-Borabicyclo[3.3.1]nonane, 9-[3 3,5-Dimethylbenzaldehyde [1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic

In the leafy vegetables screened, Quinones and saponins were found at low levels. More of phenolic compounds were observed in *Coriandrum sativum*. In *Amaranthus gangeticus*, tannins and flavonoids were seen in higher levels. Alkaloid level was very high in *Coriandrum sativum*. In both these vegetables, low level of coumarins were seen. Phytochemical levels in the organs of plants is a common effect of pest invasion and plants use these compounds as chemical weapons (Mires mailli and Isman, 2014). Tannins and flavonoids also scavenge free radicals and have larger importance in plant survival, when acting against biotic herbivores as well as pathogen and abiotic stress (pollutants). Glycosides present in these selected vegetables coincides with the work of Paul et al., 1988. This study proved that glycosides defend plants against harmful effects of herbicides by scavenging the harmful molecules. Glycosides function as potential precursors of defense metabolites which in turn gives resistance to crops.

Table 2 Distribution of certain phytochemicals in selected leafy vegetables

Phytochemicals screened	Name of the vegetables	
	Amaranthus gangeticus	*Coriandrum sativum*
Glycosides	+++	+++
Tannins	++++	+++
Flavanoids	++++	+++
Phenolic compounds	+++	+++
Coumarins	+	+
Alkaloids	+++	++++
Saponins	+	+
Quinones	+	+

The level of protein content in 1gm of *Amaranthus gangeticus* was 135±0.09 and the same in *Coriandrum* sativum was 1.22±0.05.

Table 3 Levels of protein content in selected vegetables

Sl. No.	Name of the plant	Total Chlorophyll in control vegetables (mg / gmFwt) ± S.E*	Total Chlorophyll in experimental plant material (mg / gmFwt) ± S .E*
1	*Amaranthus gangeticus*	0.87 ± 0.13	1.35 ± 0.09
2	*Coriandrum sativum*	0.73 ± 0.03	1.22 ± 0.05

*- Average mean of five replicates

Amino acid level present in selected leafy vegetables were analyzed using the method followed by Sadasivam and Manikam (1992). Amino acid present in 1gm tissue of *Amaranthus gangeticus* was 0.49±0.12 mg / gm Fwt. In *Coriandrum sativum* the amino acid level was 0.51± o.06 mg /gm Fwt.

Table 4 Amino acid levels in selected leafy vegetables

Name of the plant material	Amino Acid content control leafy vegetables (mg/gm Fwt) ± S.E*	in Proline content in experimental leafy vegetables (mg / gm Fwt) ± S .E*
Amaranthus gangeticus	0.25 ± 0.08	0.49 ± 0.12
Coriandrum sativum	0.29 ± 0.15	0.51 ± 0.06

*- Average mean of five replicates

In 1gm of *Amaranthus gangeticus* the level of proline found was 0.87 ±0.05 mg/gm Fwt, while that in *Coriandrum sativum* it was 0.67±0.16 mg/gm Fwt. High level of proline content in these crops indicate the existance of the harmful effect of harmful chemicals in these leaves.

Table 5 Levels of Proline in selected crops

Sl. No.	Name of the plant	Proline content in control leafy vegetables (mg/gm Fwt) ± S.E*	Proline content in experimental leafy vegetables (mg/gm Fwt) ± S.E*
1	*Amaranthus gangeticus*	0.57±0.17	0.87±0.05
2	*Coriandrum sativum*	0.44±0.07	0.67±0.16

*- Average mean of five replicates

As per this study, level of chlorophyll found in a gram of *Amaranthus gangeticus* was 1.56± 0.09 mg/gm Fwt. In *Coriandrum sativum* it was 1.62 ± 0.15 mg/gm Fwt.

Table 6 Level of chlorophyll in selected vegetables

Sl. No.	Name of the plant	Total Chlorophyll in control vegetables (mg / gmFwt) ± S .E *	Total Chlorophyll in experimental plant material (mg / gmFwt) ± S .E^ *
1	*Amaranthus gangeticus*	1.51 ± 0.06	1.56 ± 0.09
2	*Coriandrum sativum*	1.62 ± 0.15	1.01 ± 0.16

*- Average mean of five replicates

The carbohydrates in the selected leafy vegetables were tested by following the method used by SADASIVAM and Manikam (1992). In *Amaranthus gangeticus* the level of carbohydrate present was 1.73 ± 0.19 mg / gm Fwt. On the other

hand it was just 1.59 ± 0.07 mg/gm Fwt in *Coriandrum sativum*

Table 7 Level of carbohydrates in selected leafy vegetables

Sl. No.	Name of the plant	Carbohydrate content in control leafy vegetables (mg / gmFwt) ± S .E*	Carbohydrate content in experimental leafy vegetables (mg /gmFwt) ± S .E*
1	*Amaranthus gangeticus*	2.07 ± 0.12	1.73 ± 0.19
2	*Coriandrum sativum*	2.19 ± 0.16	1.59 ± 0.07

*- Average mean of five replicates

In the opinion of Dias[9] et al. (2004) pigment protein complexes of the photosynthetic apparatus was less stable and it degrades easily upon pesticide exposure. As chlorophyll pigments drop, the level of carbohydrate synthesized also drops in leafy vegetables taken up for this study. This may be due to the fact that pesticide interfere with the electron transport system there by affecting photosynthetic rate which in turn decrease the carbon dioxide level (Barchanska[11] et al, (2014).

SUMMMARY AND CONCLUSION

In this study the scholar has selected two leafy vegetables commonly used in the study area, Tirunelveli District, and attempted GCMS analysis and phytochemical analysis to find out the presence of pesticides and its effects. *Amaranthus gangeticus* and *Coriandrum sativum* are the two leafy vegetables selected for this study. GCMS technique proved that these vegetables contained harmful chemicals and pesticide residues. It is proved that the pesticides affected the vegetables phytochemically.

RECOMMENDATION

Crops that are grown depending on local needs that follow crop rotation by enhancing soil fertility using organic fertilizers are proved to be less susceptible to pests, disease and weed problems. Fertilizers and pesticides used after the launch of Green Revolution are proved to be harmful to human health. Vegetables must be washed thoroughly in natural extracts before use. We must join hands to grow organic vegetables to enjoy a healthy nation for the future and save our mother earth.

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