Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2014; 6(3): 550-556

ISSN: 0975-4873

Review article

Metabolic Profiling of Tomatoes with Pest Infestation Using GC-MS and NMR Spectroscopy

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Available Online: 1st September 2014

ABSTRACT
Fearly economic losses occur in the agricultural industry worldwide due to insects and pests. The monitoring of health and detection of various infections in plants is therefore critical for sustainable agriculture. Detecting early information on crop health and pest infestations can facilitate the control of these all too often devastating infections through proper management strategies such as vector control with the application of pesticides, fungicides and specific chemicals which can improve productivity. Nuclear Magnetic Resonance Spectroscopy (NMR), Gas Chromatography and Mass Spectrometry (GC-MS) analyse indicated that pest infestations significantly affect the primary as well as secondary metabolism in the plants. Here we have reviewed work of the last ten years related to metabolic profiling of tomato plant including fruit by using techniques of NMR spectroscopy and GC-MS.

Keywords: Tomato, ^{1}H NMR, GC-MS, Metabolic profiling, Pest infestation, Nanotechnology.

INTRODUCTION

The tomato is the second most important vegetable to the world in terms of total production and it has worldwide commercial distribution. The tomato is moderately rich in nutrition and is considered as an important source of vitamin A, vitamin C and minerals. Apart from this, the lycopene present in its fruit is valued for its anti-cancer properties, since it acts as an antioxidant and scavenger of free radicals[1]. Tomatoes are native to South America, but were brought to Europe sometime in the 1500s, where they became popular and subsequently were exported around the world. In tropical and sub tropical countries, the productivity and quality of tomatoes are limited by a number of constraints including biotic stresses, such as debilitating diseases and insect pests and abiotic stresses, such as high temperature, high humidity, excessive rainfall, low light intensity, and poor soil conditions[2]. In India, tomatoes are grown in an area of 634 thousand hectares with the production of 12433 thousand tons[3].

The tomato belongs under the family Solanaceae and sub family Solanum. There are currently 13 species recognized in the *Solanum* section of Lycopersicon. These species are of S. *lycopersicum, S. cheesmaniae, S. galapagense, S. pimpinellifolium, S. chmielewskii, S. habrochaites, S. neorickii, S. pennelli, S. arcanum, S. chilense, S. comeliomulleri, S. huaylasense, and S. peruvianum*[4].

Pests and Diseases in the tomato plant: Diseases are a major limiting factor for tomato production and are be classified into two groups. The first are those caused by infectious microorganisms that include fungi, bacteria, viruses, and nematodes (Table 1). These diseases are contagious and can spread from plant to plant in a field, often very rapidly when environmental conditions are favourable. The second group includes diseases caused by non-infectious physical or chemical factors such as adverse environmental factors, nutritional or physiological disorders, and herbicide injury. However non-infectious diseases may be quite extensive if an entire planting was exposed to the adverse factor.

Some common tomato pests are stink bugs, cutworms, tomato hornworms and tobacco hornworms, aphids, cabbage loopers, whiteflies, tomato fruitworms, flea beetles, red spider mite, slugs, and Colorado potato beetles[5]. *H. armigera* is a pest of many crops of major importance in most areas where it occurs, damaging a wide variety of food, fiber, oilseed, fodder, commodity and horticultural crops. Its status as a major pest is rooted in its mobility, polyphagy, high reproductive rate and diapauses, all of which make it particularly well adapted to exploit transient habitats such as man-made agro-ecosystems. Its predilection for the harvestable parts of essential food and high-value crops like cotton, tomato, pulses and tobacco confers a high economic cost to its depredations. Therefore, a high level of control is required under *H. armigera* infestations. Since in most situations the lake of adequate natural controls means that chemical, or at best integrated control methods, usually need to be adopted[6].

Detection methods for diseased and pest infected tomatoes**:** Gas chromatography and mass spectrometry (GC-MS) techniques are used for the chromatographic separation of metabolites and their mass detection. Gas chromatography (GC) coupled with MS has been used extensively in metabolite analysis because of their high separation efficiency that can resolve very complex

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Table 1. Some common diseases in the tomato plant caused by bacteria, fungi and viruses[6]

Sl. No.	Class of pathogen	Disease Center	Pathogens Center
	Bacteria	Bacterial canker	*Clavibacter michiganensis*
		Bacterial spot	*Xanthomonas campestris*
		Bacterial wilt	*Ralstonia solanacearum*
		Pith necrosis	*Pseudomonas corrugata*
		Syringae leaf spot	*Pseudomonas syringae*
	Fungi	Alternaria stem canker	*Alternaria alternate*
		Anthracnose	*Colletotrichum coccodes*
		Black root rot	*Thielaviopsis basicola*
		Black shoulder	*Alternaria alternata*
		Cercospora leaf mold	*Pseudocercosporafuligena*
		Earlyblight	*Alternaria solani*
		Fusarium wilt	*Fusarium oxysporum*
		Gray leaf spot	*Stemphylium botryosum*
		Graymold	*Botrytiscinerea*
		Southernblight	*Sclerotiumrolfsii*
	Virus	Tomatomosaic	Tomato mosaic virus
		Curlytop	*Curlytopvirus*
		Tomato mottle	Tomato mottle gemini virus
		Tomato necrosis	Alfalfa mosaic virus
		Tomato spotted wilt	Tomato spotted wilt virus
		Tomato yellow leaf curl	Tomato yellow leaf curl virus
		Tomato yellow top	Tomato yellow top virus
		Tomato bunchy top	Tomato bunchy top viroid
		Tomato plan to macho	Tomato plan to macho viroid

metabolite mixtures. In addition, it allows the easy, complete and reliable identification of compounds using an automated mass spectral deconvolution and identification system[7]. It is a low-cost method to analyze a wide range of volatile compounds and many semi volatile metabolites through chemical derivatization. The resulting GC-MS profile may characterized using the REPLIB, WILLY and NIST mass spectral library and by matching the chromatogram with appropriate standards.

NMR spectroscopy measures the resonances of magnetic nuclei such as ^{1}H, ^{13}C and ^{15}N that interact with an external magnetic field [8]. It offers non-invasive structural analysis of metabolites in crude extracts, cell suspensions, intact tissues or whole organisms allowing *in vivo* analysis[9]. NMR spectra are unique and specific for each metabolite[10, 11] and can be used to identify metabolites of biological origin, of which no a-prior knowledge is needed[12, 13]. This method provides simultaneous access to both qualitative and quantitative information and requires minimal sample preparation and is highly reproducible with a high sample throughput[14].

NMR spectroscopy is especially suitable for metabolic profiling because NMR is non-destructive we determine the chemical structures of different classes of metabolites which accumulate in response to exposure to pathogens. The most sensitive, commonly occurring magnetic isotope is ^{1}H and this is the preferred nucleus for most metabolite fingerprinting and profiling applications of NMR. Analysing the metabolic composition of a plant extract with ^{1}H NMR spectroscopy has several advantages. A crude extract can be analyzed with minimal required sample preparation. A wide range of compounds can be analyzed; it provides structural information with no volatility or polarity restrictions.

Although compromised to some extent by its sensitivity, ^{1}H NMR spectroscopy is an effective technique for both metabolite fingerprinting and metabolite profiling applications for samples of plant origin. These analysis have the potential to complement high-throughput, system-wide analysis by MS, and the application of coupled techniques that allow parallel MS and NMR analysis on the same sample would seem to be an ideal way to increase the fraction of the metabolome that can be revealed by routine analysis. The simple conclusion, however, masks the versatility of the NMR technique as a tool for metabolite analysis in at least three ways. First, it is non-destructive and spectra can be recorded from cell suspensions, tissues, and even whole plants, as well as from extracts and purified metabolites[15, 16]. Second, it offers an array of detection schemes that can be tailored to the nature of the sample and the metabolic problem that is being addressed[17]. Third, the natural abundance of some of the biologically relevant magnetic isotopes is low and this allows these isotopes, particularly ^{2}H, ^{13}C, and ^{15}N, to be introduced as labels into a metabolic system prior to the NMR analysis. The high-throughput, system-wide objective of metabolomics puts a premium on sensitivity and ubiquity; the aim is to detect as many metabolites as possible in the shortest possible time and at the lowest cost.

Here, we aimed to review the work done on metabolic profiling of tomatoes in last ten years by using spectroscopic techniques.

Metabolic profiling of tomatoes in last decade**:** The metabolic profiling of the tomato has been studied extensively in last few years. Roessner -Tunali *et al* in, 2003 studied by using GC-MS protocol alongside conventional spectrophotometric and liquid chromatographic methodologies. The metabolic profiling of transgenic tomato plants over expressing hexokinase and revealed that the influence of hexose phosphorylation diminishes during fruit development. They were able to identify in excess of 70 small metabolites and to catalogue the metabolite composition of developing tomato fruit. In addition to comparing differences in metabolite content between source and sink tissues of the tomato plant and after the change in metabolite pool sizes through fruit development, they demonstrated that the influence of hexose phosphorylation diminishes during fruit development and this highlights the importance of greater temporal resolution of metabolism[18].

The identification and quantitative analysis of metabolites, either *in vivo* or in tissue extracts, was done by Krishnan *et al*, in 2004 using NMR a spectroscopy technique. In this profiling, 20–40 metabolites in an unfractionated tissue extract were assigned by ^{1}H spectra[19].

Metabolic profiling of leaves and fruit of wild species of tomatoes. A survey of the *S. lycopersicum* complex reported by Schauer *et al*, in 2005.They found changes in metabolite contents which correlated with stress responses, as well as with metabolites of nutritional importance. These changes were discussed with respect to investigating various wild tomato species for metabolic engineering within wild breeding strategies. A total of 64 metabolites were identified in *S. lycopersicum*. The major organic acids reported were malate, citrate, succinate, various forms of ascorbate, and glycerate. Glucose, fructose, sucrose, arabinose and inositol were present in different levels in wild species of tomato plant. The domestication of the tomato *S. lycopersicum* and the associated selective pressures eventually led to the large-fruited varieties cultivated today. *S. lycopersicum* varieties are generally red-fruited, but display considerable variance in fruit colour intensity, shape and quality. The increase in productivity in cultivation is, somewhat offset by a narrowing of the crops genetic base, which has lead to an increased susceptibility to biotic and abiotic stresses. Since *S. lycopersicum* can be crossed easily with its wild species relatives, this exotic germplasm can provide a valuable source for the improvement of agriculturally important traits. A GC-MS based survey is presented here of metabolites levels of leaves and fruit of *S. lycopersicum* and five wild species of tomato that can be crossed with (*S. pimpinellifolium*, *S. neorickii*, *S. chmielewskii*, *S. habrochaites*, and *S. pennellii*) [20].

Nuclear Magnetic Resonance Spectroscopy-based metabolite profiling of transgenic tomato fruit engineered to accumulate spermidine and spermine reveals enhanced anabolic and nitrogen-carbon interactions has been done by Mattoo *et al*, in 2006. They reported nine amino acids and γ -aminobutyric acid (GABA) during ripening of wild-type, non transgenic azygous (556AZ), and two transgenic (556HO and 579HO) strains were identified in tomato fruits. The levels of isoleucine, valine, thrionine, alanine, and GABA in wild-type and 556AZ control fruit were decreased during ripening. A similar trend was apparent in 556HO and 579HO fruits, except for valine. Phenylalanine levels declined during the later stages of ripening in both nontransgenic controls (wild type, 556AZ) as well as in the 556HO and 579HO transgenic strains. However, Phenylalanine levels in the red fruit of 579HO plants were lower than in the rest of them. The levels of glutamate and aspartate decreased in the red fruit from the wild-type and 556AZ plants. Profiles of the organic acids citrate, fumarate, and malate in the wild-type and 556AZ non transgenic fruits indicated a considerable decrease in their levels after the breaker stage as ripening progressed. However, the citrate content remained significantly higher in the red fruit from both the high-polyamine transgenic lines (556HO and 659HO), although the levels of malate and fumarate declined during the ripening of the fruit from both the transgenics. Their levels in the red fruit were also significantly higher than in the azygous/wild-type controls. The higher levels of citrate, malate, and fumarate were associated with decreased glucose content in the red ripe transgenic fruits. Sucrose levels, on the other hand, decreased during ripening in the fruit from all and differences between the transgenics and non transgenics were not significant.The aspartate and hexose profiles, choline levels mostly remain similar throughout the ripening of wild-type and 556AZ fruits[21].

S. Moco *et al.*, in 2008 reported the intra- and inter-metabolite correlation spectroscopy of tomato metabolomics in data obtained by LV-MS and NMR. In this study, the metabolic profiles of ripe fruits from 50 different tomato cultivars, including beef, cherry and round types, were recorded by both ^{1}H NMR and accurate mass LC- quadrupole time-of-flight. In particular, intense signals in the sugar region, 3-6 ppm, were observed, indicating the presence of glycosylated metabolites and free sugars. From the visual comparison of the spectra, there were obvious similarities between all spectra, suggesting similar metabolic profiles in the different tomato cultivars. The analysis of (non-fractionated) tomato fruit extracts by ^{1}H NMR allowed the detection of essentially primary (polar) metabolites such as sugars, amino acids, organic acids and nucleotides, and the abundance of the corresponding resonances indicated a high natural concentration of these metabolites in the fruits. The relatively low abundance of secondary metabolites and the large amount of resonances in the spectrum made the detection of secondary metabolites, such as phenolic acids, flavonoids and alkaloids, more difficult as compared to the detection of the primary metabolites[22].

E.M.S. Pérez *et al*, in 2010, had reported the feasibility of HRMAS NMR as an efficient technique for metabolic studies of tomato fruit and tissues. HRMAS NMR spectra showed resolutions similar to that of solution ^{1}H NMR with the advantages of minimal sample manipulation and

possibility of analysing simultaneously polar and non-polar compounds. ^{1}H HRMAS NMR spectroscopy, in combination with principal component analysis (PCA) and assigned signal analysis (ASA) provided a clear differentiation between varieties as a function of the ripening process and revealed the existence of variety-dependent relationships between external and metabolic content. γ -amino butyric acid (GABA) is envisioned as a good marker for monitoring the ripening process. The content in other taste-relevant metabolites (fructose and organic acids) proved to be variety-dependent. The results show a preferential accumulation of citric acid and fructose in Rambo and Raf varieties, respectively. Raf fruits are characterized by high fructose to glucose and fructose to citric acid ratios. The content of malic acid during ripening also remains high for the Raf tomato. These combined features are responsible for the unique characteristics and exceptional taste of this traditional line of tomato[23].

K. Luengwilai *et al*, in 2011 worked on them metabolite content of the harvested Micro-Tom tomato (*S. lycopersicum* L.) by GC-MS. Using GC-MS metabolite profiling, 363 analytes were detected in the fruit pericarp, of which 65 are identified metabolites. The PCA of these data led to distinct groups among the samples based on their treatments; chilled and 'Chilled + Heat-Shocked' fruit were markedly different from each other, while the 'Non- Chilled Control' and 'Heat-Shocked' fruit were similar and grouped closer to the chilled + Heat-Shocked' fruit. These results indicate that the heat treatment provided protection from chilling in part by raising levels of fruit metabolites. The levels of arabinose, fructose-6-phosphate, valine and shikimic acid appear to be induced by this heat-shock chilling tolerance since their levels were altered in the 'Chilled' samples ($p < 0.05$), relative to the control and the heat-shocked protected fruit[24].

Miyako Kusano *et al*, in 2011 carried out studies with modified tomatoes using metabolomics for objective substantial equivalence assessment. In combination, the chosen platforms detected compounds that represent 86% of the estimated chemical diversity of the metabolites were listed in the LycoCyc database. Following a proof-of-safety approach, they showed that w92% had an acceptable range of variation, while simultaneously indicating a reproducible transformation-related metabolic signature. They were concluded that using multi-platform metabolomics is an approach that is both sensitive and robust and that it constitutes a good starting point for characterizing genetically modified organisms[25].

G. Oms-Oliu *et al*, in 2011 reported the metabolic profiling characterization of tomato fruit during preharvest development, ripening, and postharvest shelf-life. Most compounds levels in groups, showing either increasing levels (e.g., maleic and aspartic acid) or decreasing (e.g., valine and malic acid) of concentration with progressing fruit development, with some compounds being markedly characteristic for either small green fruit (e.g., shikimic acid) or fully ripened detached fruit (e.g, mannose). GC-MS was used to identify both polar and volatile metabolites that were involved in fruit development and ripening. Characteristic metabolites for the various stages of fruit development were identified. Mannose, citramalic, gluconic and keto-1-gulonic acids were shown to be strongly correlated to the final postharvest stages. During on-vine ripening, an increase was observed for the major hexoses, glucose and fructose and cell wall components such as galacturonic acid, and for amino acids such as aspartic, glutamic acid and methionine. Major changes were also observed at the level of the TCA cycle, showing a decrease in malic and fumaric acids, and an accumulation of citric acid[26].

In year 2012 M.P Lopez-Gresa *et al* reported on the metabolic fingerprinting of tomato mosaic virus infected *S .lycopersicum*.^{1}H NMR based metabolomics were the focus of this study of the compatible interaction between tomato plants and tomato mosaic virus. With this analytical platform, a total of 32 metabolites including amino/organic acids, sugars, phenylpropanoids, flavonoids and other miscellaneous compounds were detected. Using multivariate data analysis, we identified a subset of metabolites induced during the plant defence response and metabolites whose accumulation depended on developmental stage, leaf position on the stem, and harvest time. Specifically, a general time-dependent decrease in organic acids, amino acids (excluding asparagine), phenylpropanoids and rutin was observed in individual leaves. Additionally metabolite alterations to correlate with the developmental stage of the leaf: high levels of organic acids, some amino acids, phenylpropanoids, and flavonoids were found in lower leaves, while elevated amounts of sugars occurred in the upper ones. Moreover, a marked variation in the content of some metabolites was also observed to be associated with the asymptomatic ToMV infection, both in inoculated and systematically infected leaves. While flavonoids accumulated in virus-inoculated leaves, increased levels of phenylpropanoids were observed in non-inoculated leaves, where ToMV actively replicates. Finally, diurnal changes in metabolite content were also observed: an increase of amino acids and organic acids (except glutamic acid) were observed in the samples collected in the morning, whereas sugars and secondary metabolite levels were increased in the tomato leaves harvested in the evening[27].

Use of nanotechnology in controlling the pest *H. armigera*: Nanotechnology is a recent discipline to be used in pest control. The ingenuity of nanotechnology is the ability to precisely form matter to atomic level specificity. Thus, the major benefit of employing nano-based pesticides is the opportunity to enhance properties such as efficiency and specificity. The potential application and benefits of nanotechnology are enormous. These include insect pest management through the formulations of nanomaterial based pesticides and insecticides, bio-conjugated nanoparticles for slow release of nutrients and water. Nanotechnology has promising applications in nanoparticle mediated gene transfer. It can be used to deliver DNA and other desired chemicals into plant tissues for the protection of host plants against insect pests[28]. The pediculocidal and larvicidal activity of synthesized silver nanoparticles, using an aqueous leaf extract of *Tinospora cordifolia,* showed maximum mortality in the head louse

Pediculus humanus and fourth instars larvae of *Anopheles subpictus* and *Culex-quinque fasciatus*[29]. Encapsulated citronella oil nano-emulsion is prepared by high-pressure homogenization of 2.5% surfactant and 100% glycerol, to create stable droplets that increase the retention of the oil and allow for its slow release. The release rate depends upon the protection time; consequently a decrease in release rate can prolong the mosquito protection time[30]. Nanoencapsulation allows a chemical to be slowly but efficiently released to the particular host for pests control. Release mechanisms include dissolution, biodegradation, diffusion and osmotic pressure gradients with specific pH[31]. Nanopesticides, nanofungicides and nanoherbicides are used efficiently in agriculture[32]. Nanoparticles loaded with garlic essential oil are efficacious against *Tribolium castaneum* Herbst[33]. Nanotubes filled with aluminosilicate can stick to plant surfaces, while ingredients of nanotube have the ability to stick to the surface hair of insect pests and ultimately enter the body and influence certain physiological functions[34]. Some IGM techniques useful for growing tomatoes are crop rotation, resistant cultivars, seed treatment, weed control, fertilizer, irrigation, biological control and chemicals.

Integrated pest management (IPM) control: There are several methods available to control *H. armigera*. Trap cropping concentes pest population into a manageable area by providing with an area of a host crop or an area of a preferred host crop. For example the *H. armigera* population on cotton, okra and pigeon pea can be greatly reduced by growing neem as a trap crop[35]. Marigold was grown as a trap crop in tomato fields[36]. Several bio pesticides are developed against *H. armigera*. Use of neem based pesticides such as Nimikrin, Nimbidin and Nimbicidine on chickpea[37], can effectively control *H. armigera.* Some wild soybean cultivars such as 356, M4, M7, M9, Clark, Sahar, JK and BP[38] showed resistance to this pest. There are transgenic crops such as cotton[39], soybean[40], maize [41], tomato [42], cultivated throughout the world. Genetically modified cotton carrying the Cry1Ac gene isolated from *Bacillus thuringiensis*, has been incorporated into the cotton plant, which often shows resistance to *H. armigera*. Damage to the pod of pigeon pea by *H. armigera* was minimum with the application of neem seed kernel extract followed by Bt at an interval of 20 days from the pod initiation stage onwards[43]. However, GM crops have drawbacks also. GM peas made by adding a protein from beans that conferred resistance to express a Brazil nut protein was drawn from production after it was also found to be allergenic in tests[44]. There are natural chemicals emitted in the form of volatile and by virtually all known insects. Each insect species has its own unique signature scent that is a pheromone. Controlling of *H. armigera* using pheromones is very efficient and environmentally safe. The fact that only male moths were caught by pheromones and that the total number of adult moth catches was significantly higher by pheromones than by light traps was reported by Mansoor *et al*, 2012[45]. Pheromones in pest management aims at mating disruption by treating a crop with the appropriate pheromone to prevent male moths from locating "calling females" and thus suppressing mating. A principle here is the need for development of slow release formulations, which maintain relative slow release of the pheromone for several weeks, and thus disrupt mating. Trapping the male moths of target pest species and utilizing the species-specific pheromone facilitates early detection of pests occurrence. IPM is thus, more complex for the producer to implement, as it requires skill in pest monitoring and an understanding of pest dynamics, besides the cooperation of all producers for effective implementation[46].

CONCLUSION

The application and benefits of GC-MS and NMR spectroscopy are important in crops. Nanotechnology also plays a very important role. This review indicates the importance of GC-MS and NMR spectroscopy to identify the metabolites present in healthy and pest infected crops. This review also shows the importance of nanotechnology in healthy and pest infected crops.

ACKNOWLEDGEMENTS

Authors are thankful to Director, National Bureau of Agriculturally Important Insects (NBAII), Bangalore for his encouragement and support. Financial support from the Council of Scientific and Industrial Research, New Delhi, is gratefully acknowledged.

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