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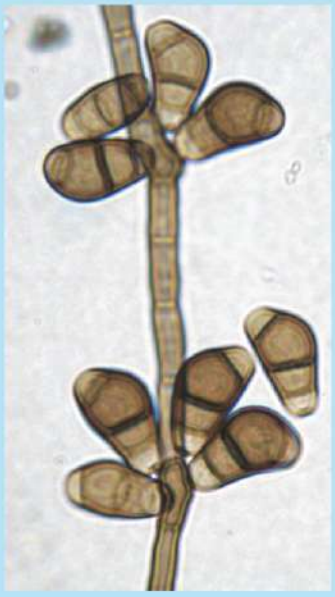

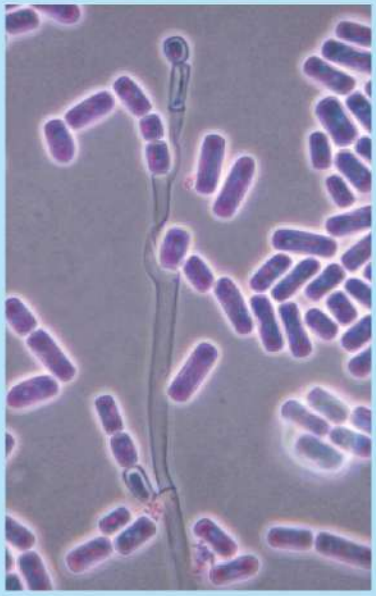

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New bioactive compounds and phytoalexins from tomato plant (*Solanum lycopersicum* L.) as red card against phytopathogenic *Alternaria cerealis* MT808477

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Abstract*:* Tomato (*Solanum lycopersicum* L.) has a source for bioactive phytochemicals. *Alternaria cerealis* is a pathogen that causes disease on variety of plant parts. This study amid to obtained novel secondary metabolites including phytoalexin compounds in tomato plant, following infection with *Alternaria cerealis* MT808477. Identification of the bioactive components present in leaves methanolic extracts was performed using gas chromatography mass spectrometry (GC-MS). Coumarin, tioconazole, octadecane, 9-ethyl-9-heptyl and fluticasone propionate were recorded. In addition, the detoxification of phytoalexin quinolizine, isoquinolizine and quinoline derivatives were also detected. Most of these compounds are candidate for valuable applications as antimicrobial, anti-inflammatory, antitumor agents and others.

Keywords: *GC-MS analysis, Tomato, phytoalexins, bioactive compounds.*

Introduction:

Tomato (*Solanum lycopersicum* L.) is very essential vegetable crop grown worldwide. In Egypt, tomato cultivation accounts for about 32% of the total vegetable growing area and total production is approximately 16% of the total vegetable output (Bekheit and Latif 2015). Egypt produces about 8 million tons of fresh tomatoes per year, making it the fifth-largest world's producer. This can be attributed to the favorable climate of the country, dual seasonality and fertile soils (Tomato News 2020). Tomatoes play a great importance in our lives because they are a source of vitamins, proteins, carotenoid (lycopene, phytoene, and b-carotene), polyphenols (flavonoids, flavanones, and flavones), carbohydrates, fibers, potassium and fats (Dahanukar *et al.* 2000 and Tan *et al.* 2010).

The studies performed showed that, tomato fruits have antimicrobial, anticancer, pharmacological and nutritional properties (Feng *et al.* 2010, Polívková *et al.* 2010 and Ahmed *et al.* 2021). The phenolic quality of tomato fruit has been correlated with its antioxidant capacity. Some compounds are also preventable oxidative shifts in cells by reducing the amount of free radicals and epidemiological studies suggest a strong link between the antioxidant ability of tomato and a reduced risk of developing cardiovascular disease and cancer (Chandra and Ramalingam 2011, Silva-Beltrán *et al.* 2015, Singh *et al.* 2017 and 2018, Ahmed *et al.* 2021 and Arfin *et al.* 2022).

Several phytopathogenic fungi of tomato attributed to the action of mainly biological and physical damage factors that affect the morphology of the fruit or its taste and smell (Etebu *et al.* 2013, Paterson and Lima 2017, Kator *et al.* 2018, Zakawa *et al.* 2019 and Hao *et al.* 2020). Among these pathogens, *Alternaria* can trigger disease symptoms in all parts of the plant (leaf blight, stem lesions and rotting fruits) and result in acute damage at all stages of plant development (Abada *et al.* 2008).

Generally, plants protect themselves against pathogenes producing many secondary metabolites such as antibiotics. Some antimicrobial catalysts called phytoalexins (Kim *et al*. 2014 and Tohge *et al.* 2014). Plants produce phytoalexins to defend against various pathogens. These toxic compounds are harmful not only to pathogens, but also to plants themselves, so plants need to be detoxified after the threat is eliminated (Camagna *et al.* 2019). Rishitin affects directly or indirectly the permeability of the plasma membrane (Lyon and Mayo 1978). The close association

between the necrosis of plants infected by microbial pathogens and the appearance of phytoalexins was discussed by Deverall (1977). Three relationships were proposed: 1) the death of the host cell contributes to the synthesis of phytoalexins in neighbouring cells; 2) the spread of invasive fungi induce phytoalexins, which can also cause cell death; or 3) the accumulated phytoalexins destroy the host cell. There are certain factors that limit or reduce the accumulation of phytoalexins, some pathogens have evolved enzymes to catabolize, transform or inhibit the synthesis of plant antitoxins, thereby overcoming their toxicity. Several fungi directly use enzymes with catabolic activity to detoxify phytoalexins (Pedras *et al.* 2011 and Zeilinger *et al.* 2016). However, *Leptosphaeria* sp. detoxifies the dithioamino group, format or reduction of the double bond of the oxindole ring (Wasalexin A) detoxified by *L. maculans* (Pedras and Abdoli 2017). Pathogens can also biotransform phytoalexins molecules.

Based on the aforementioned information, this research aims to investigate the extraction of new phytoalexins and discover biologically active compounds that developed in tomato plants as a protective means against phytopathogenic fungi through gas chromatography-mass spectrometry (GC-MS) technique.

Materials and Methods

1. Plant material

Tomato (*Solanum lycopersicum* L.) seedlings were kindly provided by Agriculture Research Center, Assiut Governorate, Egypt. The seedlings were planted in sterilized experimental soil (2 kg) and grown in greenhouse condition (approx. 20.4–36.6°C, Relative humidity 42-55%) (Sundaresan *et al.* 1993).

2. Fungal cultures

The isolate of *Alternaria cerealis* AUMC 14484 (MT808477) was used in this study which originally isolated from the infected leaves of tomato and maintained on potato dextrose agar (PDA) at 28°C.

a. Preparation of conidial suspension of *A. cerealis* MT808477

To obtain a conidial suspension (1×10^5 conidia/ml), *A. cerealis* was grown on PDA at 28°C for one week, then the conidia were collected in a sterile potato dextrose broth contains trace amounts of Tween- 80. The suspension was passed through 45 µm sieve to remove mycelial clumps (Sundaresan *et al.* 1993).

b. Pathogenicity test

Thoroughly spray the conidial suspension of *A. cerealis* on the deep wounds of the plant leaves to infect the growing tomato plants. The pots were irrigated every two days, during this period the symptomatic tomato seedlings were assessed. The plants were harvested after 48 hours and store immediately at -20°C (Tjamos and Smith 1974 and Sundaresan *et al.* 1993).

3. Extraction of phytoalexins

One gram of leaves was removed by a scissor and homogenized with liquid nitrogen. The crushed tissue has been extracted with 10 m methanol (60%) per 1 g fresh tissue. The extracts have been centrifuged at 1000 g for 10 minutes. The supernatants had been dried at 40°C. The residues were then dissolved in 10 ml methanol (60%) for gas chromatography mass spectrometry (Elgersma and Liem 1989). GC-MS analysis of the extract was performed using A tracer GC-ISQ mass spectrometer (Thermo Scientific, Austin, Texas, USA). The sample was injected into direct capillary column (30 m x 0.25 mm I.D. x 0.25 µm film thickness). The initial oven temperature was 50°C; hold for 2.0 min, to 250°C at 20 °C/min and hold for 2 min. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The injector and ion source temperature was set at 250°C. Total GC running time was 14 min. The relative percentage amount of each component in the sample was calculated, and the mass spectrums of the unknown component were compared with the spectrum of the known components stored in the National institute of Standard and Technology computer library (NIST11).

4. Identification of components

The GC-MS mass spectrum was obtained and interpreted through slanderous databases via NIST library database (National Institute Standard and Technology). The name, molecular formula, molecular weight and structure of the sample components were well known (Dubal *et al.* 2014).

Results and Discussion

The plant tissues contain a huge wealth of natural biochemical compounds that can be applied in many areas as valuable alternatives to costly and chemically manufactured products (Chaudhary *et al.* 2018, Wawrosch and Zotchev 2021). *Alternaria cerealis* AUMC 14484 (MT808477) used in the current study could estimulate the plant defense via production of several compounds after 48 h of infection. Several active principal compounds was observed in the GC-MS analysis of tomato methanol extract after 48 h, compared with untreated plant (control), of which 33 compounds have been identified. According to the basic measurement principles (retention time, RT; molecular formula, MF; molecular weight, MW and concentration or area peak, %), the predominant phytochemical compounds in methanol extract at 48 h were acids, alkaloids, flavonoids, phytoalexin compounds, glycosides, hormones, phenolic compounds, carbohydrates, fats and vitamins (Figure 1). These results were greatly similar with those obtained by Tan *et al.* (2010) and Chaudhary *et al.* (2018). They observed that tomato was a strong source of carotenoids (lycopene and carotene), phenolic, flavonoids compounds, vitamins (vitamin A and ascorbic acid) and glycoalkaloid (tomatine). On the other hand, the GC-MS analysis of the methanol extract of untreated tomato leaves resulted about seventeen identified compounds. The prevailing compounds were Phytol and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (10.69% for each), Hexadecanoic acid, methyl ester (15.31%), 9,12,15-octadecatrienoic acid, methyl ester, (47.35%). Major components present in the extract along with molecular formula, molecular weight and peak area were presented in Table (1) and Figure (2). In the respect, El Damhougy *et al.* (2017) identified twenty nine bioactive compounds from ethanol extract of *Callyspongia crassa* using GC-MS analysis, for examples, hexadecanoic acid, methyl ester, hexadecanoic acid, 14-methyl-, methyl ester, hexadecanoic acid, 15-methyl-, methyl ester, hexadecanoic acid, ethyl ester, pentadecanoic acid, 14-methyl-, methyl ester, methyl tetradecanoate, 5,8,11,14-eicosatetraenoic acid, methyl ester, (allZ) and ethyl 5,8,11,14-eicosatetraenoate.

Phytopathogenic fungi can be considered as the main infectious agents in plants, causing great alterations in plant metabolism during interaction, resulting in several bioactive chemical compounds. Accordingly, Abdel-Monaim (2017) observed an increase in activity of chitinase and β-1, 3-glucanase, phenolic, flavonoid and lignin compounds of Faba bean plants inoculated with *Fusarium oxysporum* compared to untreated plants (control).

Identification of phytoalexins in methanol extracts using GC-MS after 48 h of infection

The major phytoalexin compounds obtained from the GC-MS analysis of the methanol extract of *S. lycopersicum* L. after treatment with *A. cerealis* are the main classes of phytoalexins reported in tomato (Table 2). In this study, several phytoalexins have been recorded that belong to many plant families.

The synthesis of these compounds can protect tomato plants from external influences. Recently, five key groups of phytoalexins have been identified in Solanaceae: phenylpropanoid-related compounds, steroid glycolalkaloids, norsesqui-and sesquiterpenoids, coumarins and polyacetylene derivatives (Jeandet *et al.* 2014).

Also, tomato fruit is rich with health-promoting phytochemicals such as flavonoid, isoflavonoid, terpenoid, indole, anthraquinone and phenol compounds), that are useful in the prevention of major chronic degenerative disorders. Chaudhary *et al.* (2018) reported that tomato plants are the main source of phenolic compounds, carotenoids (lycopene, alpha and b-carotene), vitamins (ascorbic acid and vitamin A) and glycoalkaloids (tomatoine). The various parts of the tomato plant are rich

in different forms of bioactive compounds, many of which are discarded as by-products by the food industry. These compounds are potential sources of antimicrobial, antiviral, and antioxidant compounds, such as phenols, flavonoids, vitamins, and others (Silva-Beltrán *et al.* 2015, Singh *et al.* 2017 & 2018 and Ahmed *et al.* 2021 and Arfin *et al.* 2022).

The water extracts of tomato (*S. lycopersicum* L.) fruit irrigated with static magnetic field water showed the presence of carotenoids, phenols, tannins, carbohydrates and flavonoids (Dubois *et al.* 2018).

In the current results, the major components of the methanol extract of the samples along with their retention time (*RT*), molecular formula **(MF)**, molecular weight **(WF**) and area % are shown in GC-MS chromatogram (Table 3).

Detoxification of phytoalexins identified in tomato extract infected with *A. cerealis*

Phytoalexins are toxic compounds not only to pathogens but also to host plants. For example, rishitin needs to be degraded by the host after a certain period of time of infection. The compounds rishitin-M1 and rishitin-M2 are derivatives of rishitin, which have lost their toxicity to plant tissues, and at the same time, their efficacy against plant pathogenic fungi is greatly reduced. For this reason, some pathogens have evolved enzymes to catabolize, transform or inhibit the synthesis of phytoalexins, thereby overcoming their toxicity. The number of genes encoded by pathogens to reduce the toxicity of any kind of phytoalexins is still unknown (Camagna *et al.* 2019 and Westrick *et al.* 2021).

In the other hand, the detoxification of phytoalexins into non-toxic compounds was catalyzed by enzymes produced by plant pathogens. Quinoline and isoquinoline were synthesized against pathogens and inhibiting detoxification enzymes (Pedras *et al.*2017). Our results indicated the presence of some chemical compounds act as inhibitors of detoxifying enzyme of the phytoalexin belong to quinolizine and isoquinoline such as methyl 10-chloro-3(4-chlorophenyl)-6,7-dihydro-4-oxo-4h-benzo[a] quinolizine-1-carboxylate, 9h-17a,8-propeno-4h cyclododeca [4,5] pyrrolo [3,2,1-ij]quinoline-18-carboxylic acid, 5, 6, 10, 11, 12, 13, 14, 15, 16, 17- decahydro-20-oxo-,methylester, 2 (1h) –quinolinone, 5, 6, 7, 8-tetrahydro-1,3-diphenyl - 4-[(phenylmethyl)amino, 6-bromo-4- [2- [(tert-butoxycarbonyl) amino] phenyl] - 5, 8-dimethoxyquinoline, Benz[g]isoquinoline-5,10-di one, 7,9-dimethoxy-3-methyl (Table 4).

The strongest evidence for the main data on phytoalexins came from studies on the ability of certain pathogenic fungi to detoxify phytoalexins. Inducible fungal enzymes produced by plant pathogens catalyze the detoxification of phytoalexins into indole-3-carboxaldehyde and S-methyl dithiocarbamate (Pedras *et al.* 2017).

Several phytopathogenic fungi possess enzymes that can detoxify the phytoanticipins or phytoalexins released by the host. Depending on the development of plant antibiotics, the pathogens may develop a behavior that bypasses the resistance mechanism (VanEtten *et al.* 1995 and Westrick *et al.* 2021).

Brassinin is an important phytoalexin compounds produced in cruciferous plants, with phytoalexins and antibacterial activity. Several pathogenic cruciferous fungi can detoxify brassinolide. Pedras *et al.* (2009) reported that brassinin oxidase inhibitor, a phytoalexin detoxifying enzyme was produced by the plant pathogenic fungus, *Leptosphaeria maculans*.

New bioactive compounds identified in tomato leaf extract

In addition to phytoalexin compounds and some inhibitors of the detoxifying enzyme, new bioactive compounds were obtained from the methanol extract of tomato. These are tioconazole (0.19%), fluticasone propionate, diacetate of isocelorbicol (0.40%) and octadecane, 9-ethyl-9-heptyl (0.45%) listed in Table (5). The presence of various biologically active compounds in tomato leaves may be a promising source of potential antibacterial agents, and it is reasonable that tomato is

proven to be a plant with medicinal properties. In this respect, Tioconazole is a new antifungal imidazole derivative, which has significant activity against, *Aspergillus, Trichophyton* spp., *Microsporum* spp., *Pseudococcus glabrata* and *Cryptococcus neoformans* (Jevons *et al.* 1979). Bioactive ingredients present in tomato were found to have antioxidant, anti-mutagenic, anti-proliferative, anti-inflammatory and anti-atherogenic effects. Health-promoting tomatoes' bioactivity makes them a useful ingredient in the development of functional foods (Chaudhary *et al.* 2018 and Kumar *et al.* 2021).

Conclusion

Plants are good source for several valuable compounds. It is the first research to recognize new bioactive and phytoalexin compounds that are synthesized from tomato leaves after infection with *Alternaria cerealis* as a red card for phytopathogenic fungi. Using GC-MS after 48 h certain chemical compounds that act as inhibitors of the phytoalexin detoxifying enzyme were obtained. Modern analysis techniques are demand to keen several scientists to discover more and more important compounds in plant extract.

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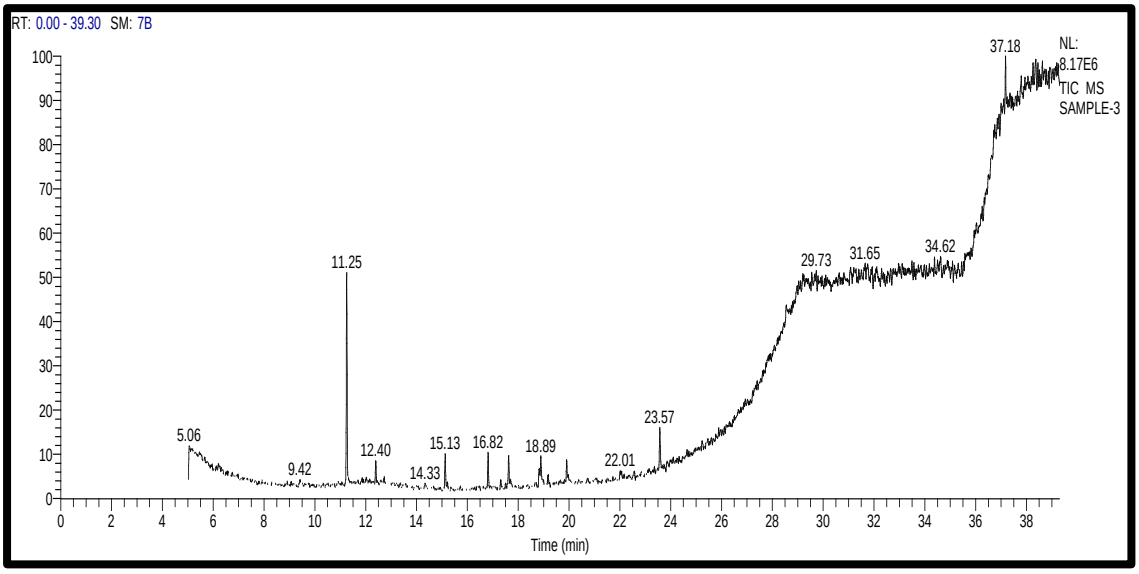

Figure 1: GC-MS spectral chromatogram of methanol extract of tomato (*S. lycopersium* L.) leaves treated with *A. cerealis*.

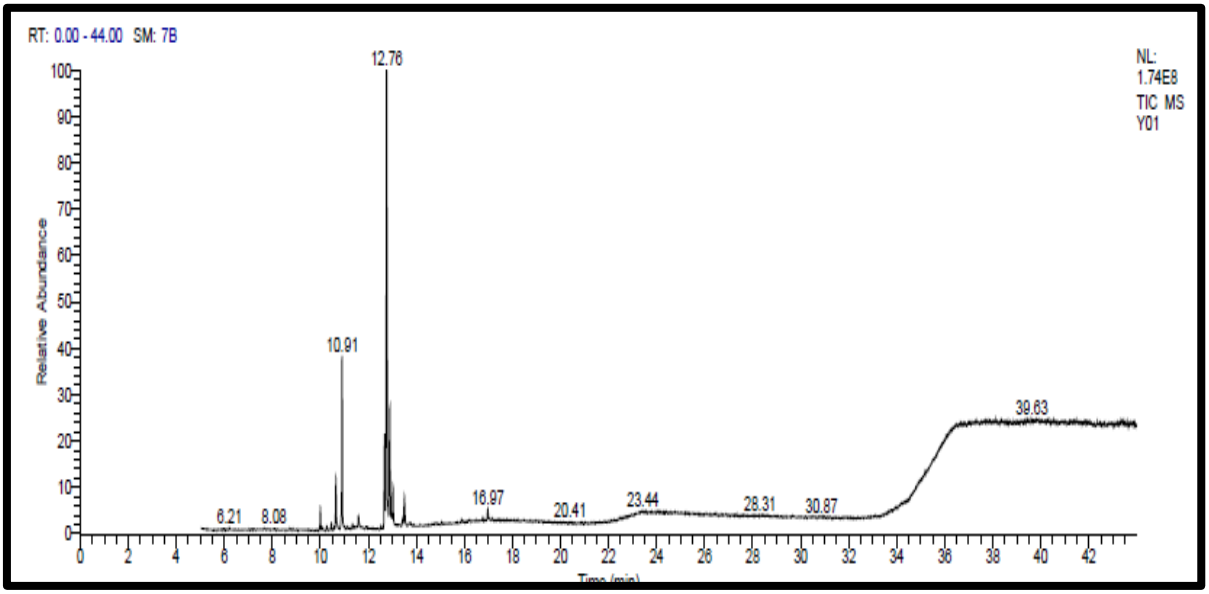

Figure 2: GC-MS spectral chromatogram of methanol extract of untreated tomato (*S. lycopersium* L.) leaves**.**

Table 1: Chemical constituents identified from the methanol extract of untreated tomato using GC-MS analysis

No.	Chemical name	RT (min)	Area%	Molecular Formula	Molecular Weight
1	4h1benzopyran4one,2(3,4dihydroxyphenyl)6,8diá dglucopyranosyl5,7dihydroxy-	16.97	1.47	$C_{27}H_{30}O_{16}$	610
2	phthalic acid, 6-methylhept2yl pentadecylester	16.97	1.47	$C_{31}H_{52}O_4$	488
3	Heptadecanoic acid, 16-methyl-, methyl ester	11.59	1.58	$C_{19}H_{38}O_2$	298
4	Neophytadiene	10.00	2.10	$C_{20}H_{38}$	278
5	2,6,10-trimethyl,14-ethylene-14-pentadecne	10.00	2.10	$C_{20}H_{38}$	278
6	Methyl stearate	13.03	3.81	$C_{19}H_{38}O_2$	298
7	Octadecanoic acid, methyl ester	13.03	3.81	$C_{19}H_{38}O_2$	298
8	Ethyl(9z,12z)-9,12-Octadecadienoate	13.51	3.63	$C_{20}H_{36}O_2$	308
9	Butyl 9,12,15-octadecatrienoate	13.51	3.63	$C_{22}H_{38}O_2$	334
10	9,12-octadecadienoic acid (z,z)-, methyl ester	13.51	3.63	$C_{20}H_{34}O_2$	306
11	7,10,13-hexadecatrienoic acid, methyl ester	10.65	5.26	$C_{17}H_{28}O_2$	264
12	Methyl octadeca-9,12-dienoate	12.68	8.79	$C_{19}H_{34}O_2$	294
13	Methyl 9-cis,11-trans-octadecadienoate	12.68	8.79	$C_{19}H_{34}O_2$	294
14	Phytol	12.89	10.69	$C_{20}H_{40}O$	296
15	3,7,11,15-tetramethyl-2-hexadecen-1-ol	12.89	10.69	$C_{20}H_{40}O$	296
16	Hexadecanoic acid, methyl ester	10.91	15.31	$C_{17}H_{34}O_2$	270
17	9,12,15-octadecatrienoic acid,methylester,(z,z,z)-	12.76	47.35	$C_{19}H_{32}O_2$	292

Table 2: Phytoalexin compounds identified from methanol extract of tomato leaves treated with *A. cerealis* by GC-MS analysis.

Types of Phytoalexins	Examples
Coumarin derivative	3,4-diphenylcoumarin
Flavonoid/ isoflavonoid	3-hydroxy-7,8,2'-trimethoxyflavone, Quercetin 3,5,7,3',4'-pentamethyl ether 3,2',4',5'-tetramethoxyflavone 5,2'-dihydroxyflavone 5,7,3',4'-tetramethoxyisoflavone 3-o-dimethylethylsilyl-5,7,3',4'-tetra-omethylquercetin 3-Hydroxy-6,2',4'-trimethoxyflavone 4h-1-benzopyran-4-one,2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy
Terpenoid compounds	2(1h)-naphthalenone, octahydro-1-methyl-1-(2-propenyl)-, (1à,4aá,8aà), 1,2,3,4-tetrahydro-5,6,7,8-tetramethoxy-1,4-methanonaphthalene Spirost8en11one,3hydroxy,(3á,5à,14á,20á,22á,25R)-
Indole compounds	-pyrido[1,2-a]benzimidazole-1,2-dicarboxylic acid,3-(4-chlorophenyl)-1,2,3,5-t etrahydro 2,5-dimethyl-3,4-bis(3-chloro-2-methylphenyl)pyrrole 1h-pyrrole, 1-bromo-2,3,4,5-tetrakis[(trifluoromethyl)thio]- 1-(4-isopropylphenyl)-2-(trifluoromethyl)-6-isopropyl benzimidazole diethyln-methyl-10h-[1]benzoselinopheno[3,2-b]indole-2,7-dicar boxylate 3,3-bis(n-methyl-3-indolyl)-n –methyloxindole
Spirobrassinin (indole phytoalexins and their derivatives	6-(t-butylimino)-8-(3',5'-dichlorophenyl)-3,4-dihydro-2h, 6h-pyrimido[2,1-b][1,3]thiazine-7-carbonitrile 6ethyl5(4'trifluoromethylphenyl)pyrimidine-2,4-diamine 5'-(trifluoromethyl)-(17r)-spiro[3á-acetoxyandrost-5-ene-17,2'(3'h)-furan]-3'-one
Anthraquinone Compound	9,9,10,10-tetramethyl-9,10-disila-9,10-dihydroanthracene 9,10-anthracenedione,1,5-dihydroxy-4,8-bis[(4-methylphenyl)amino]-
Phenol Phytoalexin	4h-1-benzopyran-4-one,2-(3,4-dimethoxyphenyl)-3,5- dihydroxy-7-methoxy-

Table 3: Phytoalexin compounds in tomato leaves infected with *A. cerealis* after 48h using GC-MS analysis.

No.	Name of compound	RT	MF	WF	Area %	Identification of the compounds
1	3,4-diphenylcoumarin	10.67	$C_{21}H_{14}O_2$	298	0.23	Coumarin
2	3-hydroxy-7,8,2'-trimethoxyflavone	7.53	$C_{18}H_{16}O_6$	328	0.20	Flavonoids
3	Quercetin 3,5,7,3',4'-pentamethyl ether	8.06	$C_{20}H_{20}O_7$	372	0.24	Flavonoids
4	3,2',4',5'-tetramethoxyflavone	9.60	$C_{19}H_{18}O_6$	342	1.19	Flavonoids
5	5,2'-dihydroxyflavone	13.24	$C_{15}H_{10}O_4$	254	0.41	Flavone
6	5,7,3',4'-tetramethoxyisoflavone	14.67	$C_{19}H_{18}O_6$	342	0.14	Isoflavone
7	3-Hydroxy-6,2',4'-trimethoxyflavone	19.45	$C_{18}H_{16}O_6$	328	0.14	Flavone
8	3-o-dimethylethylsilyl-5,7,3',4'-tetra-omethylquercetin	15.62	$C_{23}H_{28}O_7Si$	444	0.26	Quercetin
9	4h-1-benzopyran-4-one,2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy	21.40	$C_{18}H_{16}O_7$	344	0.23	Quercetin 7,3',4'-trimethyl ether
10	2(1h)-naphthalenone,octahydro-1-methyl-1-(2-propenyl)-, (1à,4aá,8Aà)-	11.25	$C_{14}H_{22}O$	939	13.49	Rishitinone
11	1,2,3,4tetrahydro5,6,7,8tetramethoxy-1,4-methanonaphthalene	15.62	$C_{15}H_{14}C_{l4}O_6$	430	0.17	Rishitinol
12	Spirost8en11one,3hydroxy,(3á,5à,14á,20á,22á,25R)-	20.73	$C_{27}H_{40}O_4$	428	0.26	Sesquiterpene, Solavetivone
13	pyrido[1,2-a]benzimidazole-1,2-dicarboxylic acid,3-(4-chlorophenyl)-1,2,3,5-t etrahydro-	8.35	$C_{19}H_{15}ClN_2O_4$	370	0.82	Indole compound as phytoalexins
14	2,5-dimethyl-3,4-bis(3-chloro-2-methylphenyl)pyrrole	12.02	$C_{20}H_{19}Cl_2N$	343	0.85	Indol alkaloids as phytoalexin
15	1-(4-isopropylphenyl)-2-(trifluoromethyl)-6-isopropyl benzimidazole	12.16	$C_{20}H_{21}F_3N_2$	346	0.69	Indole compound as phytoalexin
16	1h-pyrrole, 1-bromo-2,3,4,5-tetrakis[(trifluoromethyl)thio]-	18.27	$C_8BrF_{12}NS_4$	545	0.23	Indole compound/ as phytoalexins
17	diethyln-methyl-10h-[1]benzoselinopheno[3,2-b]indole-2,7-dicarboxylate	20.52	$C_{21}H_{19}NO_4Se$	429	0.14	Indole compound as phytoalexins
18	3,3-bis(n-methyl-3-indolyl)-n –methyloxindole	21.31	$C_{27}H_{23}N_3O$	405	0.16	indole compound as phytoalexin
19	9,9,10,10-tetramethyl-9,10-disila-9,10-dihydroanthracene	8.90	$C_{16}H_{20}Si_2$	268	0.41	Anthraquinone Compound as phytoalexin
20	9,10-anthracenedione,1,5-dihydroxy-4,8-bis[(4-methylphenyl)amino]-	21.09	$C_{28}H_{22}N_2O_4$	450	0.27	Anthraquinones act as phytoalexins
21	6-(t-butylimino)-8-(3',5'-dichlorophenyl)-3,4-dihydro-2h, 6h-pyrimido[2,1-b][1,3]thiazine-7-carbonitrile	21.72	$C_{18}H_{18}C_{l2}N_4S$	392	0.20	spirobrassinin act as phytoalexin
22	6ethyl5(4'trifluoromethylphenyl)pyrimidine-2,4-diamine	23.67	$C_{13}H_{13}F_3N_4$	282	0.43	spirobrassinin phytoalexin
23	5'-(trifluoromethyl)-(17r)-spiro[3á-acetoxyandrost-5-ene-17,2'(3'h)-furan]-3'-one	17.07	$C_{25}H_{31}F_3O_4$	452	0.40	spirobrassinin act as phytoalexin
24	4h-1-benzopyran-4-one,2-(3,4-dimethoxyphenyl)-3,5- dihydroxy-7-methoxy-	18.68	$C_{18}H_{16}O_7$	344	0.64	Phenol compound act as phytoalexin

Table 4: Some inhibitors (detoxifying enzymes) of the phytoalexin identified from the methanol extract of tomato leaves (*S. lycopersicum* L.) by GC-MS analysis.

No.	Name of compound	RT	MF	WF	area %	Identification of the compounds
1	Methyl 10-chloro-3-(4-chlorophenyl)-6,7-dihydro-4-oxo-4h-benzo[a]quinolizine-1-carboxylate	7.59	$C_{21}H_{15}C_{l2}NO_3$	399	0.10	Quinolizine derivate
2	9h-17a,8-propeno-4h-cyclododeca[4,5]pyrrolo[3,2,1-ij]quinoline-18-carboxylic acid, 5,6,10,11,12,13,14,15,16,17-deca hydro-20-oxo-, methyl ester	11.91	$C_{26}H_{31}NO_3$	405	0.26	Quinoline derivate
3	2(1h)-quinolinone,5,6,7,8-tetrahydro-1,3-diphenyl-4-[(phenylmethyl)amino]-	13.21	$C_{28}H_{26}N_2O$	406	0.39	Quinoline derivate
4	6-bromo-4-[2-[(tert-butoxycarbonyl)amino]phenyl]-5, 8-dimethoxyquinoline	14.05	$C_{22}H_{23}BrN_2O_4$	458	0.41	Quinoline derivate
5	Benz[g]isoquinoline-5,10-di one, 7,9-dimethoxy-3-methyl-	8.73	$C_{16}H_{13}NO_4$	283	0.23	Isoquinolizine derivate

Table 5: New bioactive compounds identified from tomato leaf extracts using GC-MS analysis and their biological activities

No.	Name of compound	RT	MF	WF	Area %	Bioactivity of the compound	Reference
1	**Fluticasone propionate**	7.12	$C_{25}H_{31}F_3O_5S$	500	0.40	antiallergic, antiinflammatory and antipruritic effects	**Johnson (1995)**
2	**Diacetate of isocelorbicol**	7.12	$C_{19}H_{30}O_6$	354	0.40	antifeedant and potent anti-HIV10 activities	**Duan *et al.* 1999**
3	**Octadecane, 9-ethyl-heptyl**	7.34	$C_{27}H_{56}$	380	0.45	antimicrobial	**Fouda *et al.* 2019**
4	**Tioconazole**	10.1	$C_{16}H_{13}Cl_3N_2OS$	386	0.19	antifungal medication of the imidazole class	**Kljun *et al.* 2014**