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Identification of volatile compounds from maize aerial parts infested by *Chilo partellus* (swine hoe) using GC-MS analysis

D. Pedda Kasim[1], P. Suneetha[2], M. S. R. Krishna[1*], B. Dinesh[1], R. Sri Deepthi[1], U. Lakshmi Sahitya[1]

[1]*Department of Biotechnology,* KL *University, Green Fields, Vaddeswaram, Guntur- 522502, AP, India.*
[2]*Institute of Biotechnology, Prof. Jaya Shankar Telangana State Agricultural University, Hyderabad, India*

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Zea mays is one of the most important stable foods in Asian and African countries and is the second-largest cultivated and highest-yielding crop in India. Lepidopteron stem borer *Chilo partellus* is a dangerous insect pest in maize. The stem borer makes holes on the stem and leaves and enters inside the stem for feeding. The plant was therefore investigated for its bioactive components. The present study was carried out to identify the volatile compounds present in the n-hexane extract of *Zea mays* by GC-MS analysis. Total 30 compounds were identified in both infected and uninfected samples. Among the 30 compounds butylated hydroxytoluene, n-hexadecanoic acid and 9,12,15-octadecatrienoic acid, (Z,Z,Z)- were identified as three common major components. Differences in their concentrations reveal their role in plant-insect interactions.

Keywords: *Zea mays,* GC-MS, n –Hexadecanoic acid and volatile compounds.

INTRODUCTION

Maize (*Zea mays* L.) is the third major cereal crop grown worldwide. It is extensively grown in temperate, subtropical and tropical regions of the world. Among several limiting factors, stem borer *Chilo partellus* is a serious insect pest affecting maize yield. For nutrition, the stem borer enters the host plant by forming holes on the stem and leaves. Attack of pest begins with laying eggs on leaves. Larvae then feed on leaves causing lesions. Further they bore into the stem feeding on tissues. This results in reduction in the surface area of photosynthesis in leaves. On the other side, plants possess several dynamic defense mechanisms against insect herbivores which include morphological, biochemical and molecular alterations [1]. The defense strategies against herbivores could be attained by physical barriers like trichomes, silica deposition and cell wall lignifications. Chemically, some toxic chemicals (alkaloids, terpenoids and phenolics) are synthesized by plants, which act as repellents, deterrents, antinutrients and significant part in pest control by the attracting carnivores [2, 3]. Herbivore-induced plant volatiles (HIPVs) are helpful to communicate between infected plants and natural enemies by attacking the insects, as well as they warn neighboring undamaged plants of the forthcoming danger [4]. These HIPVs help in feeding or oviposition or both of insects [5,6]. Generally HIPVs are released from the plant leaves, flowers and fruits into the atmosphere, or into the soil from the roots in response to herbivore attack [7-9]. The HIPVs are emitted by both infected and uninfected plant parts, which increases the signal detection [10].

Around 2,000 volatile compounds were reported to be released from 900 plant families, in response to herbivore attack [5]. The nature of volatile compounds produced is highly specific to the insect-plant system [11-13]. The plants exposed to HIPVs have modified levels of defense-related metabolites, for example terpenoids [14], proteinase inhibitors and phenolic compounds [15]. The main objective of the present study is to analyze the various phytochemical constituents found in the aerial parts of *Zea mays* infected and uninfected by *Chilo partellus.*

MATERIALS AND METHODS

Plants

The maize seeds were collected from the farmers' fields; the seeds were sterilized with fungicides and planted in individual pots filled with fertilized soil in an insect-proof screen house in the KL University Guntur, Andhra Pradesh. All seedlings were grown at natural conditions; when they were 4-5 weeks old, the leaves and stem were collected for GC-MS analysis.

Insects

The *Chilo partellus* moths were obtained from a mass raring unit at the Department of biotechnology, KL University. The stem borer larvae were collected and infected to the plants under closed conditions for trapping of volatile compounds.

* To whom all correspondence should be sent:
E-mail: msrkrishna81@gmail.com

Extraction of plant samples

The *Chilo partellus* infected and uninfected maize aerial parts (stem and leaves) were cleaned with tap water followed by Tween-20 and finally cleaned with distilled water; shade dried and cut in small pieces. The required quantity of these small pieces was weighed and transferred to soxhlet bags and soxhleted with n-hexane solvent until the pieces were fully immersed. Further, to remove high molecular weight compounds, waxes and heavy fractions, extracts were purified by following the method described by Rodríguez-Solana *et al.*, (2014) [16]. Treated extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis.

GC-MS Analysis

GC-MS analysis of these extracts was performed using a Thermo Fisher scientific system and a gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Xcaliber software. For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1 ml/min and an injection volume of 2µl was employed (split ratio of 10:1); Injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then at 5°C/min to 280°C, ending with a 9 min isothermal stay at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 sec and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was Xcaliber. Data handling was done by using GCMS solution software. The identification of compounds was based on the comparison of their mass spectra with those of WILEY and NIST Libraries. The name, structure and molecular weight of the components of the test materials were determined.

RESULTS AND DISCUSSION

GC MS Analysis

The n-hexane extracts of uninfected and infected maize plant volatiles were identified by GC-MS. The chromatograms of both treated and control samples are depicted in Figures 1 and 2, respectively.

From the GC-MS analysis a total of 30 compounds (Table 1) were identified in both infected and uninfected samples. Analysis revealed that in both control and treated samples, three compounds, namely butylated hydroxytoluene, n-hexadecanoic acid and 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (Table 2), displaying high peak areas, form the major components.

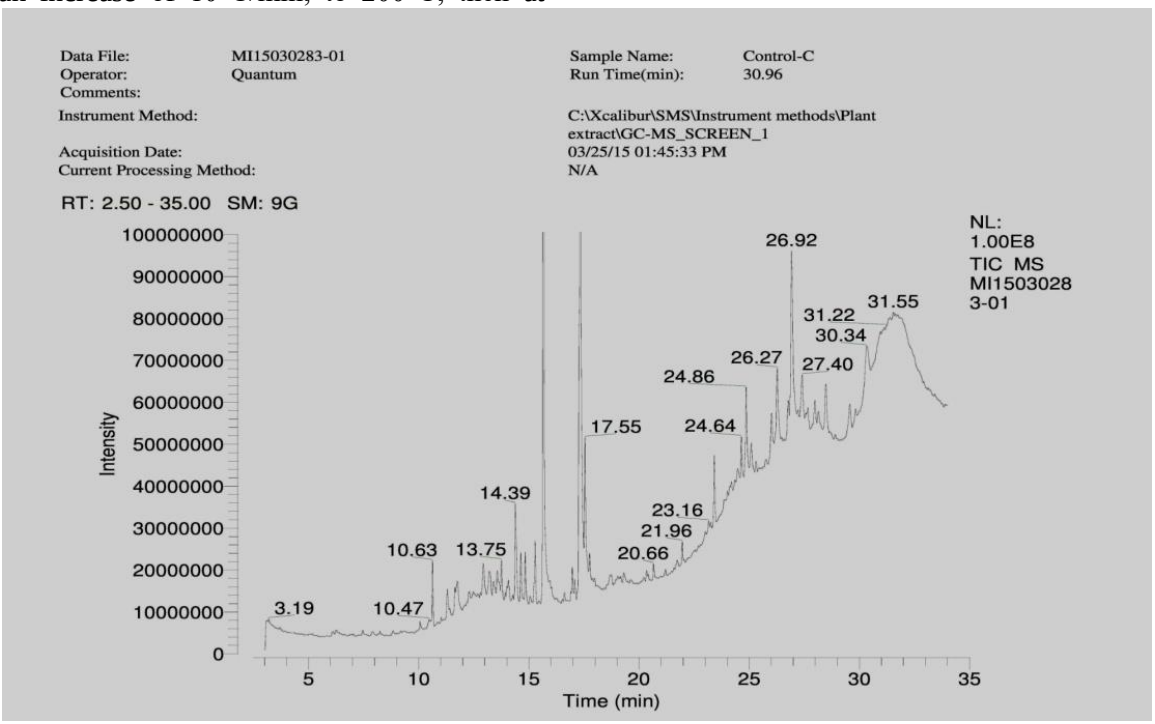

Fig. 1. GC – MS chromatogram of n-hexane extract from uninfected maize aerial parts.

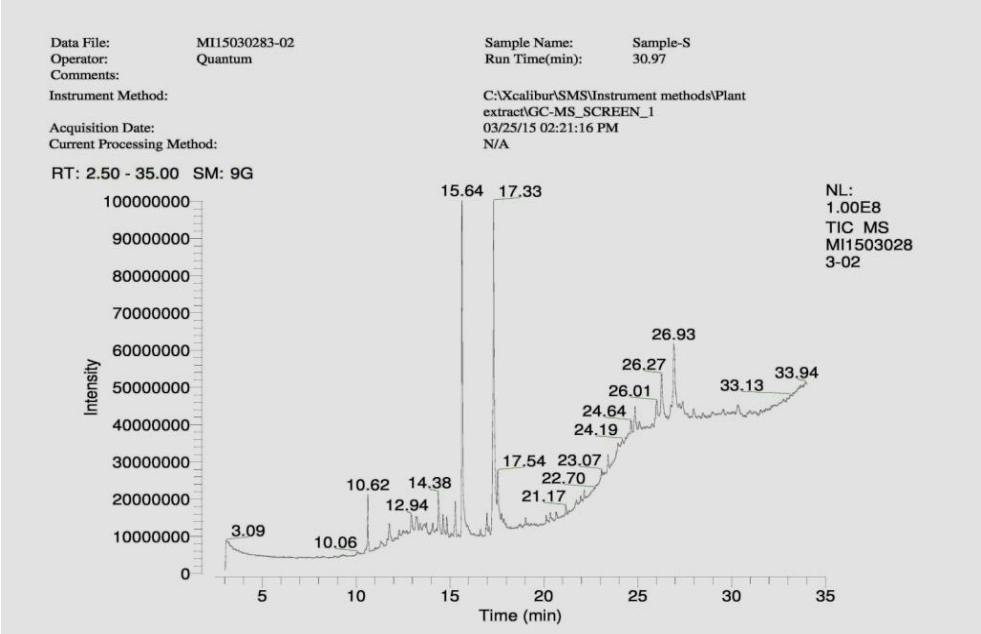

Fig. 2. GC – MS chromatogram of n-hexane extract from infected maize aerial parts

Table 1. List of volatile compounds identified in infected and uninfected maize aerial parts.

S. No	Compounds from larvae uninfected sample	Compounds from larvae infected sample
1	Tramadol	6-Octadecenoic acid, methyl ester, (Z)-
2	Dodecane, 2,6,10-trimethyl	Hexacosane, 9-octyl-
3	Butylated hydroxytoluene	Butylated hydroxytoluene
4	Dodecanoic acid	Diethyl phthalate
5	Diethyl phthalate	Tridecane, 2-methyl-
6	Dodecane, 5,8-diethyl-	Heneicosane, 11-(1-ethylpropyl)-
7	Clocortolone pivalate	Benzene, (1-butyloctyl)-
8	Dodecane, 2,6,11-trimethyl-	Benzene, (1-propylnonyl)-
9	Selenium(IV) oxide	Octadecane, 3-ethyl-5-(2-ethylbutyl)-
10	Benzene, (1-propylnonyl)-	Benzene, (1-methylundecyl)-
11	Tetradecanoic acid	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
12	2-Propenal, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester
13	Dodecane, 5,8-diethyl-	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
14	Benzene, (1-methylundecyl)-	Hexadecanoic acid, methyl ester
15	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	n-Hexadecanoic acid
16	9-Octadecen-1-ol, (Z)-	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
17	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-
18	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phytol
19	Hexadecanoic acid, methyl ester	9,12-Octadecadienoic acid (Z,Z)-
20	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
21	n-Hexadecanoic acid	Octadecanoic acid
22	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Cyclononasiloxane, octadecamethyl-
23	Phytol	1H-Indene, 1-hexadecyl-2,3-dihydro-
24	9,12-Octadecadienoic acid (Z,Z)-	Bufa-20,22-dienolide, 3,14-dihydroxy-, (3á,5á)-
25	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Phenanthrene, 9-dodecyltetradecahydro-
26	Octadecanoic acid	Spironolactone
27	1H-Indene, 1-hexadecyl-2,3-dihydro-	Lumicolchicine
28	Bis(2-ethylhexyl) phthalate	Xanthylium, 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis (ethylamino)-2,7-dimethyl-, chloride
29	Triacontane	Heptadecane, 9-hexyl-
30	Tetratetracontane	Benzene, (1-ethyldecyl)

Table 2. Major volatile compounds identified in both uninfected and infected samples

Sample	RT	Compound name	Area percentage	Formula	Molecular weight
Uninfected sample	10.63	Butylated hydroxytoluene	4.42	$C_{15}H_{24}O$	220
	15.66	n-Hexadecanoic acid	28.45	$C_{16}H_{32}O_2$	256
	17.34	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	24.23	$C_{18}H_{30}O_2$	278
Infected sample	10.62	Butylated hydroxytoluene	10.42	$C_{15}H_{24}O$	220
	15.64	n-Hexadecanoic acid	21.62	$C_{16}H_{32}O_2$	256
	17.32	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	26.84	$C_{18}H_{30}O_2$	278

Out of the three compounds, two compounds, *viz*., n-hexadecanoic acid (palmetic acid) and 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (linolenic acid) belong to the group of fatty acids, the other (butylated hydroxytoulene) being a phenol derivative. These three compounds are biologically active molecules as they play a major role in plants defense system and thence are included in the large group of protective molecules found in plants, called phytoprotectants [17].

Though the major components are the same in both samples, the concentrations of each compound varied between larvae infected and larvae uninfected samples. This shows that under the influence of herbivores, plants secrete different components with different concentrations. A higher concentration of n-hexadecanoic acid was found in larvae uninfected sample (28.55%), compared with infected sample (21.62%). The concentrations of butylated hydroxytoluene (4.42%) and 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (24.23) were identified to be low in the uninfected sample while the same compounds showed high concentrations in infected samples. Increased concentration of butylated hydroxytoluene would play a protective role in the infected sample against oxidative stress. Butylated hydroxytoluene is a phenol compound which is known to have antioxidant activity which effectively prevents oxidation [18]. Increased linolenic acid would further increase the production of pheromones by insects as insects utilize compounds released by the host plant as pheromone precursors [19]. Also, the identified compounds may act as synomones which would attract parasitoids. Hilker *et al*., 2001 [20] reported the ability of plants to produce synomones that would defend plants against the damage caused by feeding larvae.

GC-MS analysis is the initial step towards understanding the nature of plant volatile compounds. However, isolation of individual phytochemical constituents and subjecting them to biological activity analysis will definitely give fruitful results. It could be concluded that, *Zea mays* contains various bioactive compounds which are of importance in plant-insect interactions. However, further studies are needed to study its bioactivity, toxicity profile and IPM values.

CONCLUSION

In the present study total 30 compounds from the n-hexane aerial parts extracts of *Zea mays* were identified by gas chromatography–mass spectrometry (GC-MS) analysis. The nature of the identified volatile compounds is mostly fatty acids. The research findings have shown that the aerial part of *Zea mays* is extensively rich in secondary metabolites and the identified compounds can be used as synomones for controlling the maize stem borer.

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ИДЕНТИФИЦИРАНЕ НА ЛЕТЛИВИТЕ СЪЕДИНЕНИЯ ОТ НАДЗЕМНИТЕ ЧАСТИ НА ЦАРЕВИЦА, ИНФЕКТИРАНИ С *Chilo partellus*, С ПОМОЩТА НА GC-MS АНАЛИЗ

Д. Педа Касим[1], П. Сунета[2], М. С. Р. Кришна[1*], Б. Динеш[1], Р. Шри Дефти[1], У. Лакшми Сахитиа[1]

[1] Департамент по биотехнология, КЛ университет, ГрийнФийлдс, Вадесварам, Гунтур - 522502, АП, Индия
[2] Институт по биоехнология, Държавен селскистопански университет"Проф. Джая Шанкар Телангана", Хайдерабад, Индия

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(Резюме)

Царевицата (*Zea mays*) е една от най-важните храни в страните от Азия и Африка и е на второ място по култивиране и с най-голям добив в Индия. Представителите на *Chilo partellus* са едни от най-вредните насекоми за царевицата. Те пробиват дупки в ствола и листата и влизат в тях, за да се хранят. По тази причина са изследвани биоактивните компоненти в растението. В настоящото изследване са идентифицирани летливите съединения, намиращи се в н-хексанов екстракт от *Zea mays* чрез GC-MS анализ. Общо 30 съединения са идентифицирани както в инфектираните, така и в неинфектираните растения, като основните компоненти са бутилиран хидрокситолуен, н-хексадеканова киселина и (Z,Z,Z)-9,12,15-октадекатриенова киселина. Разликите в концентрациите им разкриват ролята им във взаимодействията растение-насекомо.