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Role of GC/MS analysis in early detection and poisonous compounds of infested cowpea seeds with *Callosobruchus maculatus* (Coleoptera: Bruchidae)

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Abstract

Insects cause a high loss in stored grains, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae)**.** Mylabridae is one of the most important cowpea seed (*Vigna unguiculata*) pests and causes severe damage to the stored cowpea in several regions of the world. To decrease these losses and attained of safe storage for stored grain products, there is a need to use advanced insect infestation detection methods. Based on gas chromatography-mass spectrometry (GC-MS) analysis of volatile chemical compounds (VCCs), the current work investigated the early detection of *C. maculatus* infestation in cowpea seeds and the impact of boiling (cooking) on hazardous chemicals compounds of infested cowpea seeds with *C. maculatus*. According to the findings of the GC-MS analysis, *C. maculatus* infestation caused extreme changes in the chemical constituents of cowpea seeds. Only infested cowpea seeds were ten VCCs found; boiling (Cooking) had no effect on them. Seven VCCs exposed in *C. maculatus* adults were also present in cowpea seeds affected by the same insect. These VCCs can be utilized as a marker for early *C. maculatus* infestation of cowpea seeds. Infested cooked cowpea seeds contained three hazardous and harmful chemical compounds: stigmasterol, β-sitosterol, and di-(2-ethylhexyl) phthalate. From this study, it can be inferred that the GC-MS methodology might be employed as a means of early infestation detection as well as the harmful compounds present in the infected stored grains.

Introduction

One of the most significant pests of cowpea seeds (*Vigna unguiculata*) is the cowpea weevil (Fabricius) (Coleoptera: Bruchidae). Mylabridae, which caused severe damage to stored cowpea in tropical and subtropical regions of the world (Bagheri, 1996). A few essays study the impact of boiling (Cooking process) on the volatile chemical compounds (VCCs) of infested cowpea. Insect infestation has been shown by Evangelisti *et al*. (1994) to negatively impact the chemical composition of plant foods. The main chemical components undergo significant modifications as a result of cooking (Słupski, 2010). Some toxic or non-toxic chemical compounds are produced during the cooking process as reaction products (Shamaila *et al.*, 1992).

Early detection and control of insects in the stored grains become necessary for applying corrective actions. The grain industry is very interested in applying insect-detection technologies to diminish loss percentage and enhance the goodness of grain seeds and grain-products (Laopongsit and Srzednicki, 2010). The development of this method would aim to identify VCCs that signify insect infestation. The use of GC-MS to find insect infestation in grains has been applied in incredibly few researches. Some chromatographic techniques, including gas chromatography-mass spectrometry (GC-MS), have been used to find insect volatile chemical compounds in grain products (Seitz and Ram, 2004). According to Niu *et al*. (2016), the volatile compound 2-ethyl-2,5-cyclohexadiene-1,4-dione is only present in flour infested with *T. castaneum* and it can be a helpful for the early detection of *T. castaneum* in flour. According to a recent study by Elhefny and Abdelfattah (2022), GC-MS analysis of volatile compounds can differentiate *C. maculatus*-infested cowpea seeds apart from raw or cooked cowpea seeds. Stigmasterol and beta-sitosterol, two volatile chemical compounds that can be utilized in the infestation identification of stored cowpea seeds with *C. maculatus*, were found in infected and infested cooked cowpea seeds.

Infestation of stored grains with insects is considered the most important of global problems. This infestation caused a high loss in the stored grains and changes the properties of the grains quantitatively and qualitatively, this resulting in a decline in the economic value of the grains. A stored grain infestation results in immediate damage, deterioration of grain quality, loss of feed value, and hygiene issues (Trematerra *et al.*, 2000). The interest in application of the modern insect-detection technologies has been raised in order that minimize the loss of grains and improve the quality of grain products.

Therefore, the aim of this work is to use GC-MS technique analysis of VCCs for early detection of *C. maculatus* infestation and toxic compounds in cowpea seeds.

Materials and methods

1. Infestation of seeds and insect rearing:

Cultivated adults of the tested insect, *C. maculatus*, were raised on sored cowpea seeds, *V. unguiculata* (black-eye peas) Variety Name SAMPEA 18. The seeds were stored in glass jars (Each holding about 500 ml) in the quiescent stage in accordance with the techniques of Abdelfattah and Boraei, (2017) and Elhefny and Abdelfattah (2022). Ten unsex insects per 10 g of seeds caused the infestation. The insect was reared in the Agriculture Research Centre, Dokki, Giza, Egypt's, Department of Stored Products and Grains Pests Laboratory, part of the Plant Protection Research Institute. The infected seeds were split into two groups, one of which was boiled in boiling water without dipping, and the other of which was not. Moreover, two groups of non-infested control seeds were also gathered, both cooked and uncooked. Three times each group was repeated.

2. Gas chromatography-mass spectrometry (GC/ MS) analysis:

GC/MS equipment (Fisons, Model GC8000 Series 8035 With MSD800 Mass Spectrometer Quadrupole) determined and identified the VCCs of the four treatments or samples raw (non-infested), *C. maculatus* insects (Unsexed adults), infested and cooked infested of cowpea seeds} in accordance with (Khattab *et al*., 2020). Ten seeds were included in each treatment.

Results and discussion

Study of VCCs using GC-MS

The GC-MS chromatograms of raw cowpea seeds, adults of *C. maculatus*, infected cowpea seeds, and cooked cowpea seeds with *C. maculatus* are shown in Figures (1-4), respectively.

1. Volatile chemical compounds from raw cowpea seeds *Callosobruchus maculatus* adults and *Callosobruchus maculatus* infested cowpea seeds:

According to data in Table (1) and Figures (1, 2 and 3), five VCCs were found in raw cowpea seeds, while 10 VCCs were found in infected cowpea seeds. Eleven VCCs were also found in mature *C. maculatus*. The VCCs in raw cowpea seeds changed as a result of the *C. maculatus* infestation. Only the infected cowpea seeds were found to have eight novel compounds; fresh cowpea seeds did not contain anyone.

Table (1): Volatile compounds from *C. maculatus* adults, raw cowpea, and *C. maculatus*-infested cowpea seeds were identified and determined by GC-MS.

No.	RT	Volatile compounds	Compound percentage (%)		
			Callosobruchus maculatus	Raw cowpea seeds	Infested cowpea seeds
1	6.07	2-Nitroacetophenone	ND	>1<5	ND
2	24.82	Sucrose	ND	>10<20	ND
3	38.22	Hexadecanoic acid, methyl ester	<1	>20<30	>10<20
4	39.14	Hexadecanoic acid	>10<20	ND	ND
5	42.00	9,12-Octadecadienoic acid 2-chloroethyl ester	ND	>30<40	ND
6	42.04	9,12-Octadecadienoic acid, methyl ester	<1	ND	>10<20
7	42.18	8,11,14-Docosatrienoic, methyl ester	ND	>10<20	>5<10
8	42.62	9-Octadecanoic acid (Z)-methyl ester	>1<5	ND	>1<5
9	43.19	Z-11-Hexadecenoic acid	>30<40	ND	ND
10	54.06	Nonadecane	>1<5	ND	ND
11	55.35	Pentadecane	>5<10	ND	>5<10
12	57.48	Nonacosane	>1<5	ND	>5<10
13	58.02	Triacontane	>5<10	ND	>10<20
14	58.51	Tritriacontane	>5<10	ND	>10<20
15	60.41	Cholesterol	>5<10	ND	ND
16	62.63	Stigmasterol	ND	ND	>10<20
17	63.49	Beta-Sitosterol	ND	ND	>5<10

ND= not detected
RT= Retention time

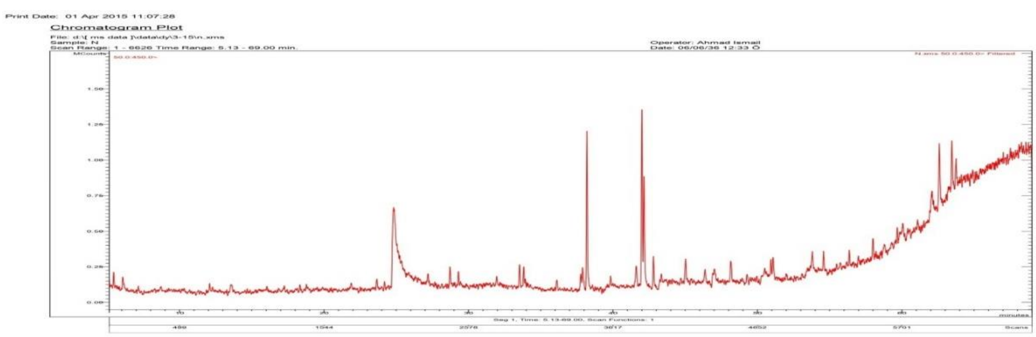

Figure (1): GC-MS chromatogram of identified volatile compounds from the raw cowpea seeds.

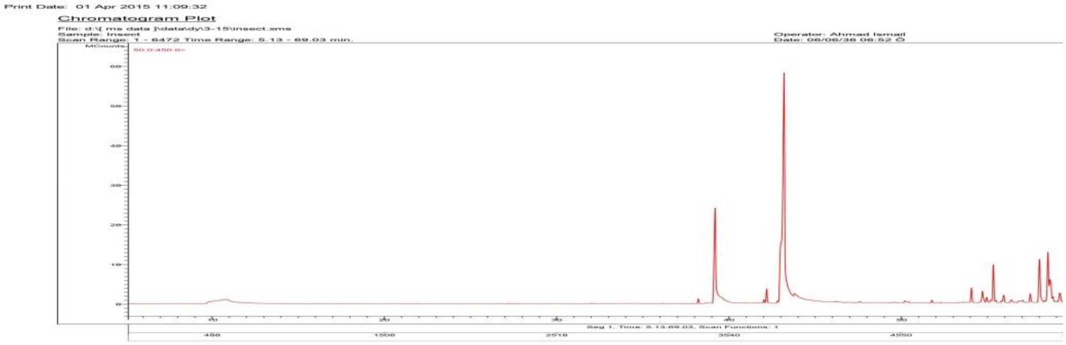

Figure (2): GC-MS chromatogram of identified volatile compounds from *C. maculatus* adults.

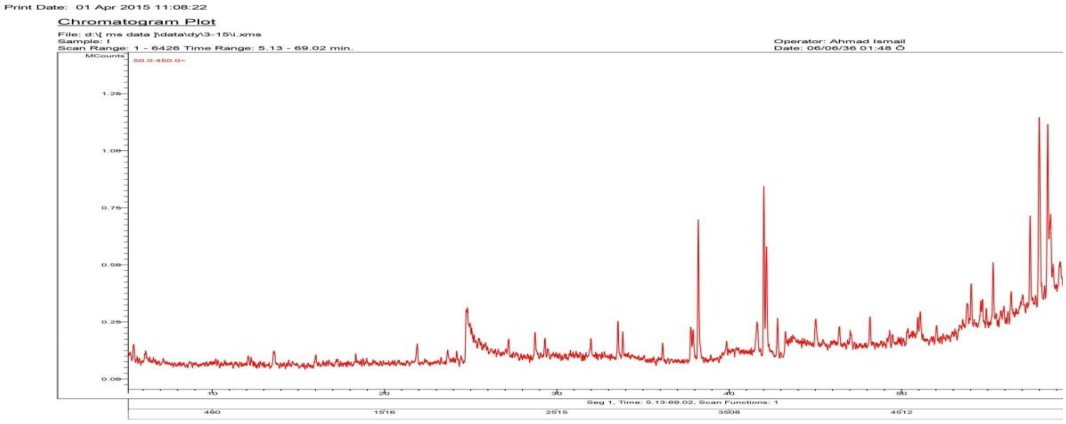

Figure (3): GC-MS chromatogram of identified volatile compounds from infested cowpea seeds.

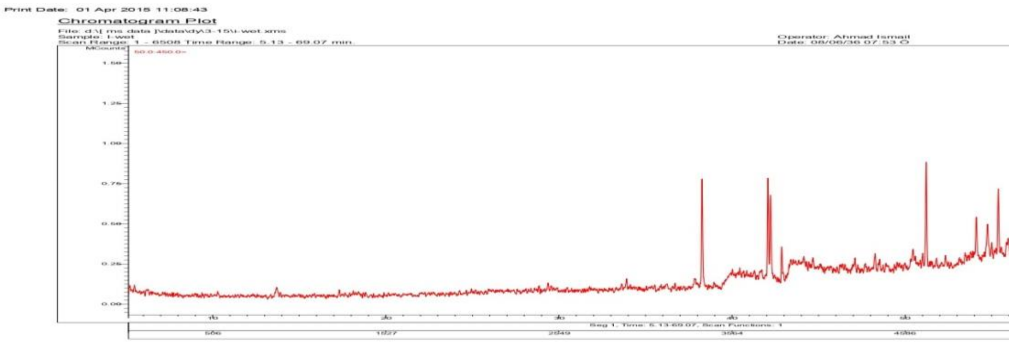

Figure (4): GC-MS chromatogram of identified volatile compounds from infested cooked cowpea seeds.

Both *C. maculatus* adults and *C. maculatus*-infested cowpea seeds contained seven VCCs: namely, Hexadecanoic acid, methyl ester; 9,12-Octadecadienoic acid, methyl ester; 9-Octadecanoic acid (Z)-, methyl ester, pentadecane, nonacosane, triacontane and tritriacontane. Cowpea seeds with *C. maculatus* infestations had larger quantities of these compounds than did *C. maculatus* adults. Detection of these seven VCCs in the infested cowpea seeds may be a sign that *C. maculatus* has infected cowpea seeds.

The presence of these VCCs may be used as of an indicator an early infestation of cowpea seeds. One volatile chemical compound (VCC), known as cholesterol, was exclusively found in *C. maculatus* adults and was not found in cowpea seeds infected with *C. maculatus*. β-sitosterol (5–10%) and stigmasterol (10–20%) were two distinct VCCs found and identified in cowpea seeds infected with *C. maculatus* alone.

2. Effect of cooking on chemical compounds of infested cowpea seeds:

Ten compounds were detected in infected cooked cowpea seeds, including seven novel compounds that were found in *C. maculatus* adults and the infected cowpea seeds, according to the results of the GC-MS analysis shown in Table (2) and Figures (2 and 3). This new compound especially two toxic compounds stigmasterol and beta-Sitosterol not affected by the cooking process quantitatively and qualitatively. On the other hand, during the cooking process, one VCC namely Di-(2-ethylhexyl) phthalate (10-15%) was only found in infested cooked cowpea seeds; it was not found in infested cowpea.

Table (2): Volatile compounds from infested and infested cooked cowpea seeds with *C. maculatus* were identified and determined by GC-MS.

No.	RT	Volatile compounds	Compound percentage (%)	
			Infested cowpea	Infested cooked cowpea
1	38.22	Hexadecanoic acid, methyl ester	>10<15	>5<10
2	42.04	9,12-Octadecadienoic acid, methyl ester	>10<15	>5<10
3	42.18	8,11,14-Docosatrienoic, methyl ester	>5<10	>5<10
4	42.62	9- Octadecanoic acid, methyl ester	>1<5	>1<5
5	51.21	Di-(2-ethylhexyl) phthalate	ND	>10<15
6	55.35	Pentadecane	>5<10	>5<10
7	57.48	Nonacosane	>5<10	>5<10
8	58.02	Triacontane	>10<15	>10<15
9	58.51	Tritriacontane	>10<15	>10<15
10	62.63	Stigmasterol	>10<15	>10<15
11	63.49	Beta-Sitosterol	>5<10	>10<15

ND= not detected

RT= Retention time

3. Effect of cooking on the toxic chemical compounds of infested cowpea seeds:

Beta-sitosterol and stigmasterol, two poisonous and significant VCCs, were found in cowpea seeds infected with *C. maculatus* but not detected in raw cowpea seeds. The act of cooking has no effect on these two VCCs. These two VCCs from the most significant phytosterols in both animals and plants. Stigmasterol and β-sitosterol are structurally related to cholesterol.

Also, a highly toxic compound called Di-(2-ethylhexyl) phthalate was detected in the cooked cowpea seeds and infested with *C. maculatus*. This means that, this highly toxic compound is not affected by the cooking process.

Infestation of stored grains with insects is a universal problem and caused a high loss in the stored grains. In order to reduce loss and improve the quality of grain and grain-derived products, there has been an increase in interest in the application of contemporary insect detecting technologies. Based on GC-MS method analysis, the goal of this work is to use VCCs to detect insect infestation and poisonous compounds in the stored grains.

Our results of the effect of infestation and cooking on the chemical composition of cowpea seeds infested with *C. maculatus* are very convenient with the results of Elhefny and Abdelfattah (2022) study who showed that, infestation and cooking caused a great change in cowpea seeds' volatile constituent. Some novel chemicals, such as stigmasterol and beta-sitosterol, which might be utilized as markers of *C. maculatus* infestations in cowpea seeds were found in infested cowpea seeds and cooked cowpea seeds infested with *C. maculatus* but not in the raw and cooked cowpea seeds. Additionally, they came to the conclusion that GC-MS can be differentiated between raw and *C. maculatus*-infested cowpea seeds. The findings of this assay were confirmed by Niu, *et al.* (2016) who found that distinct volatile chemical compounds from flour samples containing *T. castaneum* adults, healthy flour, and flour infected with *T. castaneum* were detected by GC-MS analysis. Just one volatile compound namely 2-ethyl-2,5-cyclohexadiene-1,4-dione, detected in the infested flour with *T. castaneum* and not detected with uninfected flour, this compound can be used as an indicator for the detection of *T. castaneum* in flour. De Quiros *et al*. (2000) conducted a study in which 27 chemical components were isolated from green beans and identified using GC-MS. The volatile component profile of green beans underwent significant modifications as a result of cooking. Additionally, Kermasha *et al*. (1988) investigated that all cooking methods resulted in a decrease in the amount of alcohol compounds and an increase in the amount of aldehydes and ketones in the French bean.

The inclusion of phytosterol esters in foods, which have the effect of lowering cholesterol levels, has boosted interest in phytosterols (Stigmasterol and β-sitosterol) in recent years (Berger *et al*., 2004). Studies in the field of toxicology have shown that stigmasterol and β-sitosterol have negative effects and lead to phytosterolemia (cardiovascular disease). Tao *et al*. (2019) demonstrated that the accumulation of stigmasterol, one of the phytosterol species, results in cardiac interstitial fibrosis, macrophage infiltration, and increased mortality without atherosclerosis. A stronger cardiac hazardous compound than β-sitosterol and campesterol, according to Tao *et al*. (2019), is stigmasterol. Significant mortality and stigmasterol accumulation are mostly correlated with cardiac damage. It is not well understood how stigmasterol results in heart fibrosis. In a different investigation, Malini and Vanithakumari (1990) demonstrated that β-sitosterol changed serum cholesterol levels in a way that had a tendency to have a hypocholesterolemic effect.

The two secondary metabolites of phytosterols, stigmasterol and β-sitosterol, are not toxic and pose no risk to human health, according to other toxicological investigations. In addition to being non-genotoxic, non-cytotoxic, and non-carcinogenic, stigmasterol and β-sitosterol had no long-term toxic consequences (Aguilar *et al*., 2012 and Li *et al*., 2012). Human liver cancer cells can be treated with β-sitosterol and stigmasterol, which can cause apoptosis (Li *et al*., 2012). Both in vivo and in vitro, the stigmasterol had an inhibitory effect on hepatoma cells (Zhang *et al*., 2008).

On the other hand, after cooking infested cowpea seeds, the highly toxic and extremely hazardous compound namely, Di-2-ethylhexyl phthalate (DEHP) was found in cowpea seeds. The findings of Lara *et al*. (2018), which showed that phthalate esters (PAEs) are present in maize grain and flour, supported the existence of DEHP in cowpea seeds. In additional research, di-(2-ethylhexyl) phthalate (DEHP) was found in soil and wheat grains, as demonstrated by Lü *et al*. (2018) and Shi *et al*. (2019). The PVC films and plastic containers used to cover and store food are made of phthalate esters (DEHP). Pollutants from the degradation of plastic films, such as phthalate esters (PAEs), could be constantly discharged into the soil and even accumulation in grains, endangering human health and food safety (Shi, *et al.*, 2019). In soils (Lü *et al*., 2018 and Wang *et al*., 2015), vegetables (Fu and Du, 2011 and Wang *et al*., 2015), grains (Cai *et al*., 2015), and cereals, (Schecter *et al*., 2013 and Lu *et al*., 2016), PAEs have been widely found. DEHP is heavily utilized in our daily life despite being extremely poisonous and dangerous to organism health. Exposure to DEHP has been demonstrated to have a number of negative consequences for both people and animals. DEHP has been found in several kinds of food and can enter the human body and hence causes its toxic hazards via several routes like ingestion; inhalation and also through intact skin (Ji *et al*., 2014 and Liu *et al*., 2017). The developmental toxicity of DEHP has also been investigated, and this includes impacts on brain development, intrauterine death, developmental delay, and morphological deformities and variants (Environmental Protection Agency, 2009). Results of GC-MS analysis of this work indicated that, the presence of seven volatile chemical compounds in the infested cowpea seeds can be used as an indicator to an early infestation of cowpea seeds with *C. maculatus*. Consequently, the volatile isolation technique (GC-MS) could be used as a monitor of *C. maculatus* in stored grains. Two toxic compounds (β-sitosterol and stigmasterol) were only detected in the infested cowpea seeds samples and not affected by the cooking process, while infested cowpea seeds showed that the presence of highly toxic compound namely Di-2-ethylhexyl phthalate during the cooking process. Therefore, the cooking process of cowpea seed does not completely remove of the toxic compounds. These findings call the concern and attention for the pollution of grains crops in soil or plastic containers with some toxic compounds. According to this study must be aware of consuming infested or cooked infested cowpea seeds.

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