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# Identifying flavor compounds in pollen and their role in attracting honeybee

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## *Abstract*

### Objective

Bee pollen is an essential source of nutrition for honeybees. In addition, bee pollen has biological properties such as antioxidant, antibacterial, antifungal, immune modulator, antitumor, anti-aging, anti-anemia and anti-osteoporosis. This study was conducted to identify the diversity of flavor compounds in bee pollen and their effect in attracting worker honeybees within the colony.

### Materials and methods

Three types of bee pollen were collected from honeybee colonies during different seasons using pollen traps installed at the hive entrances. The extraction of flavor compounds from bee pollen grains was carried out. Gas chromatography-mass spectrometry (GC-MS) was performed on a fused silica capillary column with specifications. The completely randomized design (CRD) was used for data analysis.

### Results

The results showed a diversity in flavor compounds in the three types of bee pollen and a difference in the proportions of their components. Type A of bee pollen was unique in that it contained its own flavor compounds that were not available in the other two types, B and C, and vice versa. Several compounds were detected at similar retention times in the three pollen types, while these compounds differed in their proportions according to the type of bee pollen grain. Among the most important compounds that appeared in the three types of bee pollen grains were 9,12-octadecadienoic acid (Z,Z), n-hexadecanoic acid, 4H-pyran-4-one, γ-sitosterol, linolenic acid derivatives, and campesterol. In addition to similar compounds that appeared in small proportions. Regarding the effect of flavor compounds in attracting worker honeybees while feeding on them, the results of the statistical analysis ($P \leq 0.05$) showed no significant differences between the averages for the three types of bee pollen.

**Conclusion**

Our results highlight the importance and utility of pollen flavors in bee nutrition and behavior. The more we understand about the chemical composition of bee pollen and its role in honey bee attraction, the better we can help improve artificial diets and pollen substitutes used in beekeeping practices.

**Keywords:** attraction, bee pollen, honeybees, flavor compounds

**Paper Type:** Research Paper.

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## Introduction

Bee pollen is collected from floral sources by worker honeybees and carried in pollen baskets (corbiculae) located on their hind legs, which is used to feed bee larvae. Bee pollen is characterized by containing a diverse combination of active substances such as proteins, amino acids, some enzymes, and fatty acids, in addition to various vitamins, minerals, and polyphenols (Almeida-Muradian et al., 2005; Birben et al., 2012). The pollen that bees collect from floral sources is much better than those which humans collect directly from floral sources. Because, honeybees selectively collect pollen from nutritionally valuable floral sources available to them, which are usually of two types. The first is Entomophilous pollen, which bees accept and collect and which has great nutritional benefits. The second type is Anemophilous pollen, that bees stay away from and do not collect and which has a role in irritating allergies (Mazza et al., 1999). Honeybees collect their food from different types of floral sources. The botanical origin of pollen plays an important role in determining its chemical composition, which directly affects its sensory properties. The plant source and the area where the food was collected also affect its components (Machado et al., 2020). Bee pollen is an essential source of nutrition for honeybees. In addition, bee pollen has biological properties such as antioxidant, antibacterial, antifungal, immune modulator, antitumor, anti-aging, anti-anemia and anti-osteoporosis (Fratellone et al., 2016; Lau et al., 2022; El Ghouizi et al., 2023). Flavor organic compounds are emitted mainly from floral sources, leaves, fruits, pollen, and other reproductive parts, and they mainly help to attract various pollinating insects, including honeybees (Loreto & Schnitzler, 2010; Hirata et al., 2016). The availability of natural nectar and pollen becomes scarce for honeybees during dry and rainy seasons in some areas, as honeybees cannot obtain them due to seasonal changes. Therefore, beekeepers must intervene directly in the nutrition of honeybees by adding suitable and inexpensive feed substitutes and supplements to keep the beehives from dying during those seasons (Al-Mana & Al-Yafrsi, 2021; Amera et al., 2024). Plants and some living organisms, such as insects, produce many organic chemical compounds. They consist of a substance or a mixture of substances. These chemical compounds stimulate behavioral or physiological responses between individuals of the species or between different species of insects. Responses between insects towards the plant occur due to the effect of flavor compounds in the plant, which affects the attraction of insects towards food sites, mating sites, egg-laying sites, and shelters (Witzgall et al., 2010). Worker bees ferment pollen grains and convert them into bee bread. In this case, bee bread surpasses the original pollen grains due to the synthesis of new compounds in them, which means that its nutritional value has become higher compared to pollen grains. Regardless of the plant origin of the pollen grains used in nutrition, fermentation affects the increase in the nutritional value of the pollen grains (Roulston & Cane, 2000; Zheng et al., 2014; Bryś et al., 2021). Although pollen substitutes are consumed and palatable by worker honeybees, their nutritional value cannot be determined until they are evaluated through nutritional testing. The most important of which is verifying the efficiency of the food source of pollen substitute meals that bees feed on and determining the actual benefit that bees obtain from pollen substitutes or nutritional supplements (Tapia-Rivera et al., 2025). The aim of this study was to identify the main flavor compounds present in pollen grains and evaluate their efficiency in attracting worker honeybees to the hives.

## Materials and Methods

**Pollen sampling:** Three types of bee pollen were collected from honeybee colonies during different seasons using pollen traps installed at the hive entrances. The traps allowed the collection of pollen loads carried on the hind legs of worker honeybees.

**Pollen cleaning and storage:** The collected pollen was first cleaned using a series of hand sieves. This was done to remove impurities and debris. A fiberglass sieve was used to remove fine dust particles. A mesh sieve with a pore size of 3 mm was used to remove larger debris.

Samples of the three pollen grain types were kept in airtight opaque plastic bags at −18 °C until testing could be carried out.

**Preparation of ethanolic bee pollen extract:** Ethanolic extracts of pollen were prepared based on the modified method of Oroian et al. (2020). One gram of pollen was mixed with 10 mL of 70% ethanol. After that, it incubated at 40 °C for 4 h. then, this mixture was centrifuged at 5000 rpm for 5 min. The supernatant was filtered and stored in opaque glass vials at 4 °C until GC-MS analysis.

**GC-MS analysis:** Gas chromatography was performed for the analysis according to the method of Graikou et al. (2011). A GC-MS instrument supplied by Agilent Technologies, Santa Clara, CA, USA, connected to an Agilent 5975 C mass selective detector. Chromatographic separation was performed on a fused silica capillary column with specifications (30 m × 0.25 mm I.D., 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was set at 200 °C, and the oven temperature was programmed to increase from 100 °C to 300 °C at a rate of 5 °C/minute. Two microliters of the sample were injected in split mode (1:20). The temperature of the transfer line and ion source was maintained at 280 °C. Scan mode was used to determine the retention time and the sample mass fragmentation signature. The mass selectivity detector (MSD) was used to identify the compounds by comparing the mass spectra in this study with those obtained from the NIST 05 Mass Spectrometry (MS) library. Compounds with a similarity index higher than 80% were considered identified. The abundance of flavor compounds in the sample was calculated as a percentage of the total peak area.

**Attractiveness test for worker honeybees:** The three types of pollen were included in the bee feed in flat plastic containers inside the beehives, with 50 grams of each type of pollen. The containers were weighed every 24 h for three consecutive days, and the difference between the readings for each type of pollen was recorded.

**Statistical Analysis:** The completely randomized design (CRD) was used for data analysis. Mean comparisons were performed using the least significant difference (LSD) test at $P \leq 0.05$ using SPSS software version 25 (IBM Corp., Armonk, NY, USA).

## Results and discussion

**Flavor compounds in pollen separated by GC-MS technology:** The results in Figure 1 show a group of flavor compounds in pollen type A taken from beehives identified using gas chromatography - mass spectrometry (GC-MS) technology. 58 flavor compounds were observed. The major compounds detected were 9,12-octadecadienoic acid (Z,Z), n-hexadecanoic acid, 4Hpyran-4-one, γ-sitosterol, linolenic acid derivatives, and campesterol. Other compounds have appeared in different proportions.

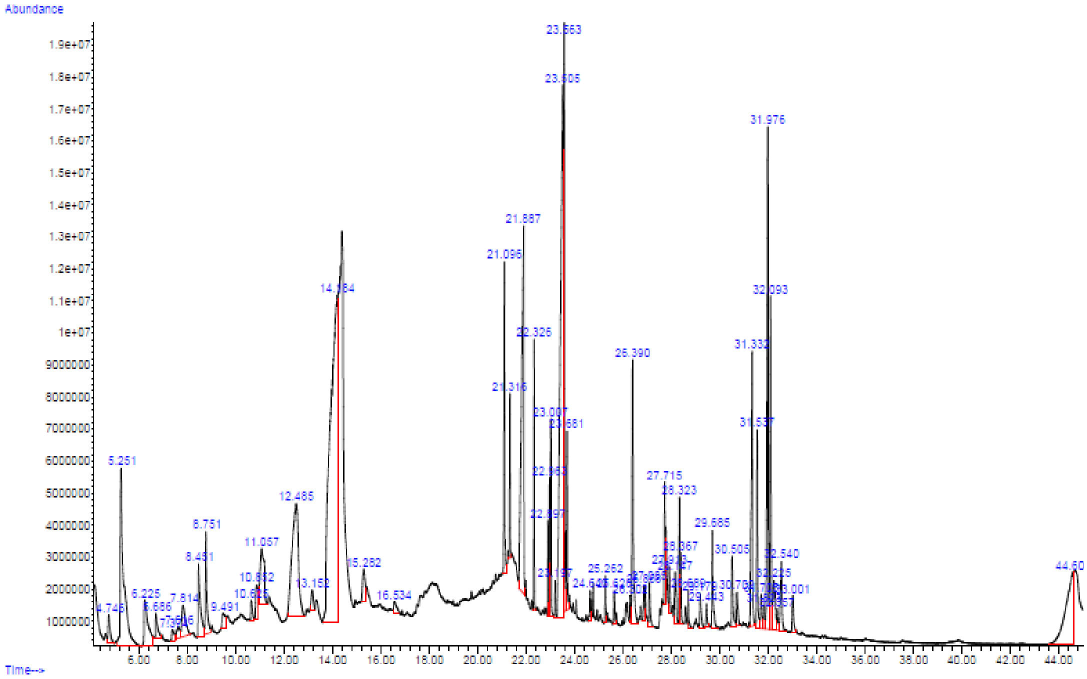


**Figure 1. Flavor compounds in Pollen Type -A- Identified by GC-MS**

Figure 2 shows a mixture of flavor compounds in type B pollen identified by GC-MS. Forty-two flavor compounds were identified. The most important of which were 9,12,15-Octadecatrienoic acid, (Z, Z, Z), n-Hexadecanoic acid, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-, Androst-5,15-dien-3ol acetate, Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z, Z, Z)-), Ergosta-7,24(28)-dien-3-ol,4-methyl-, 3.beta.,4.alpha.,5.alpha, and 16-Pregnen-3,20-dione. Moreover, with the appearance of other compounds of different types in varying proportions. The distinctive flavors and smells of some chemicals can benefit both plants and animals, and the benefit is mutual between them. Examples include smells that attract pollinating insects. Moreover, the smells of some flavor plant compounds can repel some insects (Turlings et al., 1990). Figure 3 shows a group of flavor compounds in type C pollen identified by GC-MS. The appearance of 45 compounds is noted. The most important of which were 9,12,15-Octadecatrienoic acid, (Z, Z, Z)-, n-Hexadecanoic acid, Pregna-5,16-dien-20-one, 3-hydroxy-, (3.beta.)-, Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z, Z, Z), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester), Stigmasta-5,24(28)-dien-3-ol, 3.beta.,24Z, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, and Pyridine, 2,6-diethyl. Along with other compounds in proportions shared by the three types of pollen grains. At studying mono- and polyfloral Moroccan bee pollen samples through various tests conducted to determine the diversity and quantities of individual flavor compounds, as well as their phenol and flavonoid content.

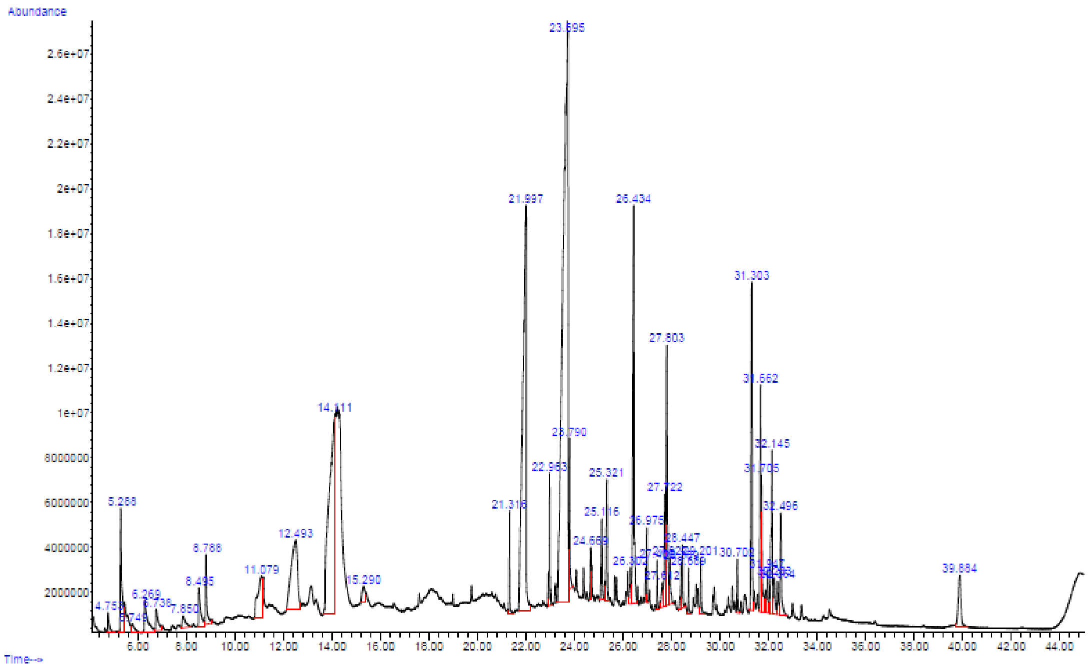


**Figure 2. Flavor compounds in Pollen Type -B- identified by GC-MS**

Many differences were found between mono- and polyfloral bee pollen samples in terms of diversity and concentration of bioactive compounds. The results of the study contributed to setting quality standards for bee pollen and promoting the consumption of this natural product from beehives (Aylanc et al., 2023).

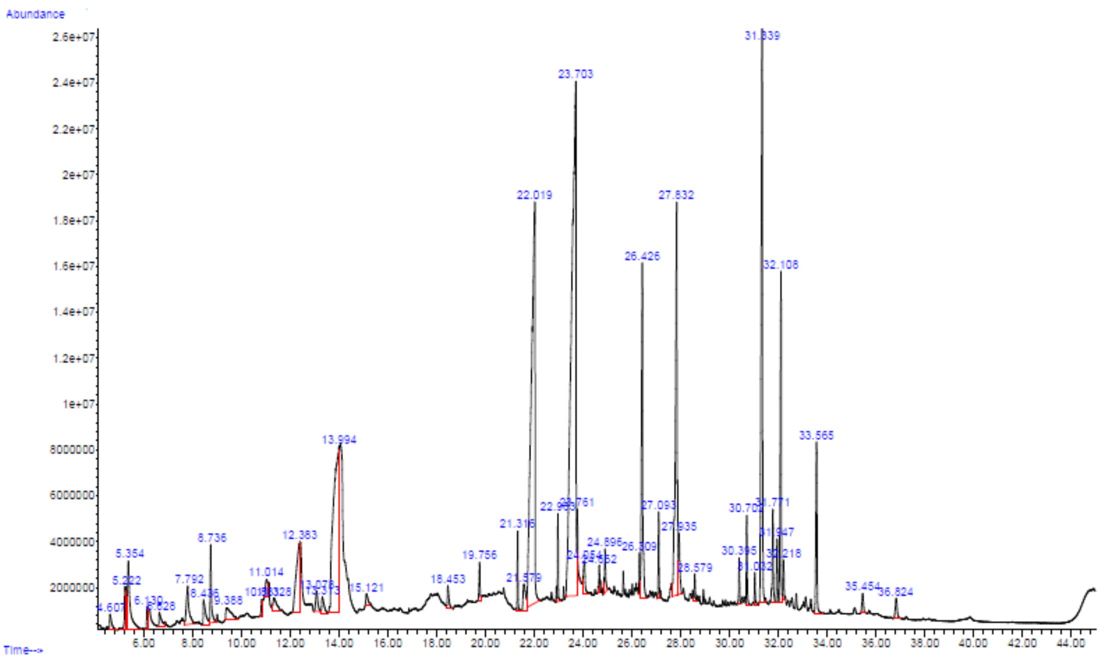


**Figure 3. Flavor compounds in Pollen Type -C- identified by GC-MS**

The chemical composition of pollen varies depending on the botanical origin, which vary according to the plant source. The color of pollen also varies according to the floral source visited by the bees (Blasco et al., 2004; Corvucci et al., 2015). Fresh bee pollen contains a certain percentage of moisture, making it a good source for microbial growth. Therefore, bee pollen samples must be dried before storage. Oven drying can alter its chemical composition, which can negatively affect its nutritional value. Compared to freeze-drying technology in terms of flavor and free fatty acid content, these compounds decreased significantly in oven-dried pollen samples in terms of their protein, fat, and total phenol content. Accordingly, freeze-drying is the best method for taking bee pollen samples to preserve their nutritional value (Keskin & Özkök, 2020). Table 1 shows the flavor compounds identified in the three types of bee pollen (A, B, and C) based on GC-MS analysis. These types shared the same flavor compounds that appeared at the same retention time, despite the different proportions in the three types of pollen grains; they have a prominent role in attracting worker honeybees.

**Testing the attraction of flavor compounds in pollen to worker honeybees:** Figure 4. shows the effect of flavor compounds on attracting worker honeybees within honeybee colonies during feeding on pollen. Statistical analysis showed significant differences in pollen consumption among the time periods ($P \leq 0.05$). It was found that after 24 hours of placing the pollen feed, the bees consumed 30.66% of the pollen of type A, 37.34% for type B, and 39.55% for type C. After 48 hours, the amount of pollen consumed increased from type A to 59.61%, type B to 51.52%, and type C to 56.92%. The percentage of pollen consumed after 72 hours was 90.27%, type A pollen was 88.86%, and type C was 96.47% consumed. There were no significant differences between the three pollen types in terms of bee attraction, as well as the overlap between the types of pollen and time periods.

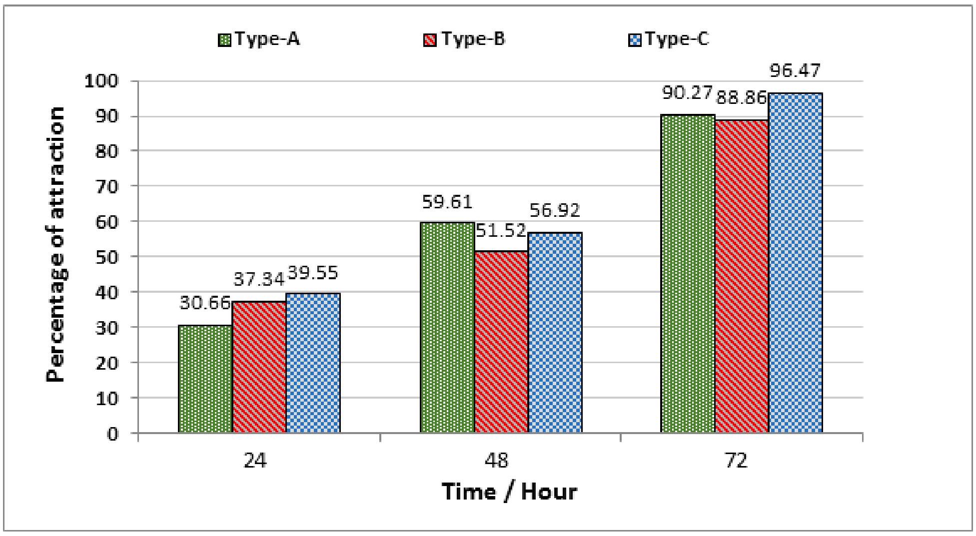


**Figure 4. Attraction of flavor compounds in pollen by worker honeybees**

**Table 1. Extracted and identified flavor compounds obtained from bee pollen**

| RT | Chemical name | Chemical structure | GC response A | GC response B | GC response C |
|---|---|---|---|---|---|
| 4.74 | Acetamide, N-(3,5-dichlorophenyl)-2-(1-pyrrolidinyl)- | C12H14Cl2N2O | + | + | + |
| 5.25 | 3,5-Dimethylpyrazole | C5H8N2 | + | + | + |
| 6.22 | Methylenecyclopropanecarboxylic acid | C5H6O2 | + | + | + |
| 7.61 | Dodecane, 1-fluoro- | C12H25F | + | - | + |
| 8.45 | 2-Furancarboxaldehyde, 5-methyl- | C6H6O2 | + | + | + |
| 8.75 | 3-n-Butylthiolane | C8H16S | + | + | + |
| 9.49 | .epsilon.-N-Formyl-L-lysine | C7H14N2O3 | + | + | - |
| 10.23 | Aziridine, 2-methyl-2-(2,2,4,4-tetramethylpentyl)- | C12H25N | - | - | + |
| 13.15 | Uridine, 5-methoxy- | C10H14N2O7 | + | + | + |
| 13.30 | cis-2-Butyl-5-(4-hydroxybutyl)pyrrolidine | C12H25NO | + | + | - |
| 14.36 | 4-Mercaptophenol | C6H6OS | + | + | - |
| 18.09 | 14-Pentadecenoic acid | C15H28O2 | + | + | + |
| 19.45 | Tetradecanoic acid | C14H28O2 | + | - | - |
| 19.73 | l-Gala-l-ido-octonic lactone | C8H14O8 | + | + | + |
| 21.31 | Hexadecanoic acid, methyl ester | C17H34O2 | - | - | + |
| 21.88 | n-Hexadecanoic acid | C16H32O2 | + | + | + |
| 22.06_ | 2-Myristynoyl pantetheine | C25H44N2O5S | + | - | + |
| 22.32 | 3,7,11,15-Tetramethylhexadeca-1,6,10,14-tetraen-3-ol | C20H34O | + | - | - |
| 22.50 | Estra-1,3,5(10)-trien-17.beta.-ol | C18H24O | - | + | - |
| 22.96 | Oleic Acid | C18H34O2 | + | + | + |
| 23.00 | 2-Nonadecanone | C19H38O | + | - | - |
| 23.68 | Octadecanoic acid | C18H36O2 | + | + | + |
| 24.66 | 1-Heptatriacotanol | C37H76O | - | + | + |
| 25.32 | Dasycarpidan-1-methanol, acetate (ester) | C20H26N2O2 | - | + | - |
| 26.39 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | C19H38O4 | + | + | + |
| 27.76 | 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester | C21H38O4 | + | + | + |
| 27.91 | Octadecanoic acid, 2,3-dihydroxypropyl ester | C21H42O4 | + | + | + |
| 28.14 | E, E, Z-1,3,12-Nonadecatriene-5,14-diol | C19H34O2 | + | - | + |
| 28.32 | Octadecanoic acid, octyl ester | C26H52O2 | + | + | + |
| 29.68 | Eicosanoic acid, n.-octyl ester | C28H56O2 | + | + | - |
| 30.50 | Ethyl iso-allocholate | C26H44O5 | + | + | + |
| 30.70 | Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5.beta.)- | C29H50O2 | + | + | + |
| 31.33 | Campesterol | C28H48O | + | + | + |
| 32.09 | Stigmasta-5,24(28)-dien-3-ol, (3. beta.,24Z)- | C29H48O | + | + | + |
| 32.22 | Stigmasterol | C29H48O | + | + | + |

Commercial pollen alternatives available in markets that do not contain any trace of pollen used by beekeepers, especially those containing soybean flour, were less efficient at attracting than the original pollen (Manning et al., 2007; Moayed Saffari, 2008). The response of insects to plants with different flavor substances either attracts or repels them. This characteristic varies according to the diversity of insects. The classification of plant flavor substances as attractants or repellents varies according to the insect's response to them and their transformation in the plant, as well as according to their concentration in it (Kirk et al., 2021).

**Conclusions:** In this study, the flavor compounds present in three types of honey bee pollen were investigated and their role in worker bee attraction was assessed. A diverse range of flavor compounds were identified in the pollen samples using gas chromatography-mass spectrometry (GC-MS) analysis. These include fatty acids, sterols and aromatic compounds. A total of 58 compounds were detected in pollen B, 42 compounds in pollen A and 45 compounds in pollen C. The most important of them are n-hexadecanoic acid, 9,12-octadecadioic acid derivatives, linolenic acid derivatives, campesterol and stigmasterol. These are the compounds that play a role in creating the specific aroma of pollen. Although the three pollen types differed in terms of the number and relative abundance of flavor compounds, several compounds were common among them. This indicates that there is a common chemical profile between them that is related to their plant origin. These flavor compounds may play an important role in mediating interactions between plants and pollinators. The uptake experiment showed that worker bees readily consumed all pollen types tested. Furthermore, their consumption increased over time. However, no significant differences were observed between pollen types in terms of their attractiveness to bees. These findings suggest that flavor compounds in bee pollen may play a role in its attractiveness and acceptance by worker bees. Overall, our results highlight the importance and utility of pollen flavors in bee nutrition and behavior. The more we understand about the chemical composition of bee pollen and its role in honey bee attraction, the better we can help improve artificial diets and pollen substitutes used in beekeeping practices.

## Author contributions

M. A. S. contributed to preparing the research plan, collecting samples, and testing bee attraction. M. S. H. contributed to analyzing the flavor compounds in pollen grains. G. F. A. contributed to the statistical analysis, collecting references of the manuscript.


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**Data availability**

The data that contributed to the completion of this study was provided by the researchers in this manuscript.

**Ethical considerations**

No human participants or laboratory animals were used in the study, and all steps were carried out in laboratories according to standard instructions.

**Conflict of Interest**

The authors declare that there is no conflict of interest with respect to this manuscript.

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# شناسایی ترکیبات فرّار در گرده و نقش آن‌ها در جذب زنبور عسل

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## چکیده

**هدف:** گرده زنبور عسل منبع غذایی بسیار مهمی برای زنبورهای عسل است. علاوه بر این، گرده زنبور دارای ویژگی‌های زیستی متعددی مانند خاصیت آنتی‌اکسیدانی، ضدباکتریایی، ضدقارچی، تعدیل‌کننده سیستم ایمنی، ضدتومور، ضدپیری، ضدکم‌خونی و ضدپوکی استخوان است. این مطالعه با هدف شناسایی تنوع ترکیبات فرّار در گرده زنبور عسل و بررسی تأثیر آن‌ها در جذب زنبورهای کارگر در داخل کلنی انجام شد.

**مواد و روش‌ها:** سه نوع گرده زنبور عسل در فصل‌های مختلف از کلنی‌های زنبور عسل با استفاده از تله‌های گرده که در ورودی کندوها نصب شده بودند جمع‌آوری شد. استخراج ترکیبات فرّار از دانه‌های گرده زنبور انجام گرفت. سپس آنالیز ترکیبات با استفاده از دستگاه کروماتوگرافی گازی–طیف‌سنجی جرمی (GC-MS) و ستون مویین سیلیکای ذوب‌شده انجام شد. برای تحلیل داده‌ها از طرح کاملاً تصادفی (CRD) استفاده گردید.

**نتایج:** نتایج نشان داد که در سه نوع گرده زنبور تنوعی از ترکیبات فرّار وجود دارد و نسبت اجزای آن‌ها با یکدیگر متفاوت است. نوع A گرده زنبور دارای ترکیبات فرّار اختصاصی بود که در دو نوع دیگر (B و C) مشاهده نشد و بالعکس. چندین ترکیب در زمان‌های نگهداری مشابه در هر سه نوع گرده شناسایی شدند، اما نسبت آن‌ها بسته به نوع گرده متفاوت بود. از مهم‌ترین ترکیبات مشاهده‌شده در هر سه نوع گرده می‌توان به 9,12-octadecadienoic acid (Z,Z)، n-hexadecanoic acid، 4H-pyran-

4-one، γ-sitosterol، مشتقات linolenic acid و campesterol اشاره کرد. علاوه بر این، ترکیبات مشابه دیگری نیز در مقادیر کم مشاهده شد. در مورد تأثیر ترکیبات فرّار در جذب زنبورهای کارگر هنگام تغذیه، نتایج تحلیل آماری ($P \leq 0.05$) نشان داد که بین میانگین‌های سه نوع گرده تفاوت معنی‌داری وجود ندارد.

**نتیجه‌گیری:** نتایج این پژوهش اهمیت و نقش ترکیبات فرّار گرده در تغذیه و رفتار زنبورهای عسل را نشان می‌دهد. هرچه شناخت بهتری از ترکیب شیمیایی گرده زنبور و نقش آن در جذب زنبورهای عسل داشته باشیم، می‌توانیم به بهبود جیره‌های مصنوعی و جایگزین‌های گرده در مدیریت و پرورش زنبور عسل کمک کنیم.

**کلمات کلیدی:** ترکیبات فرّار، جذب، زنبورهای عسل، گرده زنبور

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