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Design and evaluation of Polymer Carriers for extracted pheromone traps targeting termites (*Microcerotermes diversus* Silvestri)

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ABSTRACT

The termite *Microserotermus diversis* Silvestri is one of the destructive pests that invade fruit tree trunks, including date palms, in central and southern Iraq. This necessitates the development of environmentally safe control methods. This study aimed to assess behavioral control methods against the termites infesting date palms. An alcohol extraction process isolated components from the female glands for chemical analysis using gas chromatography-mass spectrometry (GC-MS). The effectiveness of these extracts in attracting male alates was evaluated in the laboratory to identify the optimal concentration for attracting males for mating. The most effective concentration (150 microliters) was loaded onto two types of polymer-carriers: cellulose fibers and paraffin wax. Fourier-transform infrared spectroscopy (FTIR) analysis confirmed no chemical reactions between the polymers and pheromones, indicating physical mixing only. Field trials were conducted in a termite-infested palm orchard (1 hectare) in Amara, southern Iraq, from late March to early April 2024. Traps containing cellulose-based pheromone carriers demonstrated the highest attraction for winged male termites, with an average of 8.667 males per trap after seven days. This study highlights the potential of pheromones as a targeted and sustainable approach for termite control.

تصميم وتقييم مصائد فيرمونية ومواد بوليميرية حاملة تستهدف حشرة الارضة (*Microserotermes diversus* Silvestri)

عقيل عبد الرزق كريدي ، غسان عيسى مشحوت، عقيل عدنان اليوسف، مسلم عاشور العطبي
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الخلاصة

يُعد النمل الأبيض (*Microserotermus diversis* Silvestri) من الآفات المدمرة التي تغزو جذوع أشـــجار الفاكهة، بما فيها نخيل التمر، في وسط وجنوب العراق. وهذا يستلزم تطوير أساليب مكافحة آمنة بيئيًا. هدفت هذه الدراسة إلى تقييم أساليب المكافحة السلوكية للنمل الأبيض الذي يصيب نخيل التمر. تم عزل مكونات من الغدد الأنثوية الجنسية باستخدام عملية استخلاص بالكحول و التحليل الكيميائي باستخدام كروماتوغرافيا الغاز-مطياف الكتلة .(GC-MS) و تقييم فعالية هذه المستخلصات في جذب ذكور النمل الأبيض المجنح في المختبر لتحديد التركيز الأمثل لجذب الذكور للتزاوج. تم تحميل التركيز الأكثر فعالية (150 ميكرولتر) على نوعين من حاملات البوليمر: هي ألياف السليلوز وشمع البارافين. أكد تحليل مطيافية الأشعة تحت الحمراء (FTIR)عدم وجود تفاعلات كيميائية بين البوليمرات الحاملة والفيرومونات، مما يشير إلى عدم وجود تغير كيميائي في المجاميع الفعالة للمستخلص الفيروموني وان الاختلاط فيزيائي فقط. أجريت تجارب ميدانية في بستان نخيل موبوء بالنمل الأبيض (هكتار واحد) في مدينة العمارة، جنوب العراق، من أواخر مارس إلى أوائل أبريل 2024. أظهرت المصائد التي تحتوي على حاملات فيرومون مكونة من السليلوز أعلى جاذبية لذكور النمل الأبيض المجنح، بمتوسط 8.667 ذكرًا لكل مصيدة بعد سبعة أيام. تسلط هذه الدراسة الضوء على إمكانات الفيرومونات كنهج امن ومستدام لمكافحة النمل الأبيض في بساتين النخيل.

الكلمات الافتتاحية: الفيرومونات، الارضة، الأفراد المجنحة ، البوليمرات الحاملة، تحليل مطيافية الأشعة تحت الحمراء (FTIR)

INTRODUCTION

Pheromones play a key role in insect communication and developing new pest control methods. Pheromones influence insect behavior in directional movement, aggregation, defense, and reproduction (Abd El-Ghany, 2020). In termites, numerous termite pheromones have been identified they play significant roles in essential activities and the organization of social living within termite colonies, these pheromones are important for foraging, colony defense, sanitation, and caring for the reproductive caste and eggs.(Eggleton, 2010; Mitaka & Akino, 2021).The majority of research efforts have concentrated on two types of pheromones in termite: trail-following pheromones and sex-pairing pheromones (Sillam-Dussès *et al.*, 2011), Despite the existence of numerous other pheromones in termites, particular attention has been devoted to exploring the chemical composition and functional properties of sex pheromones (Verma *et al*., 2009).

These sex pheromones are used by the winged individuals during the mating behavior period (Lo and Watanabe 2011). Following an annual or biannual dispersal event from the colony, depending on some factors such as colony age, activity, and prevailing warm and humid climatic conditions (Abdulkader *et al.* 2011). The winged termite alights on the ground or nearby trees; Subsequently, it goes through wing shedding and actively searches for a mate (Bordereau & Pasteels, 2010). In some terrestrial species, female termites undergo wing shedding and adopt a characteristic posture by elevating the abdominal region while emitting pheromones from specialized glands located in abdominal segments in the tergal or sternal region to attract males for mating. Notably, in termite species such as *Zootermopsis nevadensis*, *Z. angusticollis* both sexes, female and male, release pheromones for mating purpose (Bordereau *et al.*, 2010). Sexual mating pheromones have been identified in 17 terrestrial termite species belonging to three families (Archotermopsidae, Rhinotermitidae, and Termitidae). These pheromones primarily consist of aliphatic aldehydes, alcohols, and binary terpenes (Lo & Eggleton, 2010). In some terrestrial families, the emission of pheromones differs between males and females release (5E)-2,6,10-trimethyl-5,9-undecadienal, while males release 4,6-dimethyldodecanal (Bordereau et al., 2011). In the families Termitidae and Rhinotermitidae, only females are responsible for releasing pheromones, which predominantly comprise (Z, Z, E)-3,6,8-dodecatrien-1-ol in several species, although some terrestrial species use a mixture of two or three compounds for example, in *Odontotermes formosanus*, two pheromone compounds work synergistically over long distances, while the compound (Z, Z)-3,6-dodecadien-1-ol acts at close range (Wen *et al*., 2012).

The composition of sexual mating pheromones varies across different termite species, for instance, in *Cornitermes bequaerti* only (Z, Z, E)-3,6,8-dodecatrien-1-ol is used, while C. cumulans uses both compounds (Z, Z, E).)-3,6,8-dodecatrien-1-ol , (E)-nerolidol. On the other hand, *C. silverstrii* uses a mixture consisting of three compounds, (Z, Z, E)-3,6,8-dodecatrien-1-ol, (E)-nerolidol, and (Z)-3- dodecen-1-o). Additionally, there are other compounds present in low concentrations, which are called (pre-existing pheromones) that help in identifying sympatric species (Allison & Cardé, 2016; Chen et al., 2018; Valterová et al., 2019).

The use of slow-release pheromones in termite control enhances the effectiveness of pheromone traps. This controlled release mechanism ensures that the pheromones remain effective for a longer duration; this system provides a more efficient and sustainable method for managing termite populations (Atterholt *et al*., 1998). Numerous carrier materials, especially polymers, have been evaluated for their ability to control the release of pheromones. Polymer Carriers are designed to degrade slowly, ensuring a steady release of pheromones. The choice of polymer, its thickness, and its permeability are crucial factors in determining the release rate (Brezolin *et al.*, 2018).

Atterholt *et al*.(1999) suggested paraffin wax and aqueous paraffin emulsions could be employed as controlled release carriers of insect sex pheromones. In the laboratory, a continuous release of the pheromone of the oriental fruit moth *Grapholita molesta* from paraffin emulsions was detected for over 100 days; the release rates ranged from 0.4 to 2 mg/day depending on the dried emulsion's surface area and concentration. Hellmann *et al.*(2011) indicate to possibility of manufactured nanopolymers from cellulose acetate, Additionally, they found that pheromones could be released from the cellulose fibers over several weeks in a fairly linear form.

Moreover, slow-release pheromone traps offer an environmentally friendly alternative to traditional chemical pesticides. By targeting termites specifically, these traps minimize the impact on non-target species and the environment. This approach aligns with integrated pest management (IPM) strategies that emphasize sustainable and ecologically sound methods of pest control. The goal of the study is to develop polymer carriers for pheromone traps aimed at controlling termite infestations in date palms.

MATERIAL AND METHODS.

Laboratory studies

Termite colony

Termite colonies were collected during the period of increased population density(out break) of termite at the beginning of October to December. The base of fronds infested with termites in palm orchards were cut and placed inside a plastic box (20 * 30 * 15) cm. These samples were brought to the laboratory, where they were acclimatized, and maintained under conditions of temperature and humidity (25±1C 0 and 75±3% RH). Winged individuals were subsequently selected to conduct the rest of the experiments.

Extraction of termites pheromones

For extracting pheromones, a modified method based on (Raina et al., 2003; Wen et al., 2012, 2015) was used. Alate individuals were selected from the colony. Both females and males, which have the sternal and tergal glands, were selected and collected in a small container.

A total of 250 females were isolated as in Figuer 1 A. The distinction between males and females is based on the sternite of the last abdominal rings, which are elongated and wide compared to the males. The last abdominal tergite and sternite were cut out using a sharp scalpel and clamping forceps as in Figuer 1 B . Next it was placed in a small can , 5 ml containing 80% hexane.

The extract was sent to the Laboratory of Food Science and Consumer Protection, at College of Agriculture, to diagnose the chemical compounds by GC-MS.

A

B

Figure1: A:termite alate B: Female of termite alate (disecting of last sternal and tergal segments)

Laboratory assessment of the extracted termite pheromone

An experiment was conducted to evaluate the alcoholic extract of the tergal and sternal of the last abdominal segments (XIII-X) at the laboratory of Agriculture College, Department of Plant Protection / Insect Sciences Laboratory. The bioassay was done depending on the modified method of (Bordereau et al., 2011).

Two plastic boxes, each with dimensions of 10 cm x 10 cm x 5 cm, were connected with small plastic tubes. In one box, three concentrations of the extract (50, 100, and 150 microliters) were prepared and placed on 2 cm x 2 cm filter paper squares. The filter papers were left for one day to allow the hexane to volatilize. This setup was used to determine the appropriate concentration for field evaluation. Data were collected over 24 and 48 hours. The values were converted to the percentage of insects attracted to the filter paper moistened with pheromone using the equation below.

$$\%\ \text{Alates males attracted} \quad \frac{Number\ of\ insects\ attracted}{total\ number\ of\ insects} *100\%$$

Design of Polymer Carriers for extracted termite pheromone

Using paraffin as carrier materials for pheromone extract.

The pheromone extract was loaded onto paraffin wax following the modified method described by (Atterholt *et al*., 1999):

Ten grams of pure paraffin wax was melted on a hot plate at 60°C. One hundred microliters of the extract were added using a micropipette, with constant stirring. The heating process was stopped. The mixture was poured into disk molds until the paraffin solidified. The prepared disks were stored in a refrigerator at 4°C until applied in the orchard.

Using date palm fibers (Cellulose, Hemicellulose) as carrier materials for pheromone extract.

The pheromone extract was loaded onto date palm fibers Cellulose and hemicellulose depending on the modified method of (Campion and Lester 1978; Hellmann and Wendorff 2011):

A dry palm frond was collected and cut into small pieces, then ground using a grinder (Silver Crust grinder, Germany). Ten grams of the powder were added to 10 ml of ethyl alcohol and 5 ml of 70% sulfuric acid. The mixture was heated on a hot plate at 80°C for 30 minutes. Next, the alcohol and acid were removed using filter paper (Whatman #1). Finally, the date palm powder (fiber) was used to incorporate the pheromone extract.

Fourier Transform Infrared Spectroscopy (FTIR) analysis:

Fourier Transform Infrared Spectroscopy (FTIR) analysis confirmed no reaction between the extract, cellulose fibers, and paraffin. These reactions could result in chemical changes altering the composition of the pheromone extract compounds or forming new active groups.

Field evaluation for Termite pheromone traps

The field evaluation experiment was conducted in a private palm orchard infested with termite located in Amara city (**Figuer 2 A)** (coordinates N31.794896, E47.195648), from late March 2024 to mid-April 2024; the experiment coincided with the swarming period of termites. Jackson traps (**Figuer 2** B) were utilized in the experiment. Four sets of traps were used: (1) pheromone release systems based on polymer paraffin wax, (2) pheromone release systems based on polymer cellulose fibers (selected based on optimal concentration determined in laboratory evaluations), (3) traps containing only paraffin, (4) traps containing only cellulose fibers, and (5) control treatment (empty traps). Numbers of the alate termites caught in each trap were recorded weekly and analyzed statistically.

A B

Figuer 2: A: Distribution of traps in the orchard B: Jackson trap.

Statistical analysis :

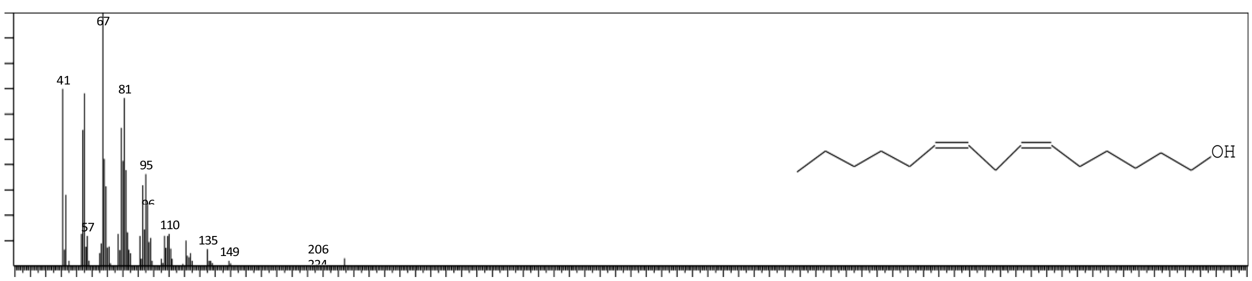
The data were tested using analysis of variance (ANOVA) and means were compared using a Daunken test at $P \leq 0.05$ for the field experiments and 0.01 for the lab, by using the IBM SPSS statistical program, version 25.

RESULTS AND DISSCASIONS

The results of laboratory analysis, using GC-MS technology to identify chemical compounds, showed the presence of 20 chemical compounds (Table 1). These included aromatic compounds, fatty acids, and esters. Cis-Vaccenic acid was the highest concentration among all the compounds; it is followed by (Z)6, (Z)9-Pentadecadien-1-ol, which is one of the simple un-saturated alcohols.

Table 1: Chemical compounds in alcoholic extract of abdominal sternal and tergal glands.

Peak#	R.Time	Area	Area%	Name
1	3.210	550028	3.69	Pentadecafluorooctanoic acid, tridecyl ester
2	11.147	169982	1.14	Hexadecane, 1,1-bis(dodecyloxy)-
3	11.464	208585	1.40	4-Estren-4,17.beta.-diol-3-one, tri-trimethylsilyl
4	12.671	92851	0.62	N-(Trifluoracetyl)-O,O',O"-tris(trimethylsilyl)epinephrine
5	15.061	1096263	7.35	n-Hexadecanoic acid
6	17.143	1724829	11.56	Linoelaidic acid
7	17.246	2889953	19.38	cis-Vaccenic acid
8	17.583	256689	1.72	Octadecanoic acid
9	17.987	163733	1.10	Hexadecanoic acid, butyl ester
10	19.339	312681	2.10	Glycidyl palmitate
11	20.309	485323	3.25	Linoelaidic acid
12	20.428	527662	3.54	n-Propyl 11-octadecenoate
13	20.514	167297	1.12	Octadecanal
14	21.775	2629462	17.63	(Z)6,(Z)9-Pentadecadien-1-ol
15	21.885	2428464	16.28	Glycidyl palmitoleate
16	22.304	266729	1.79	Glycidyl palmitate
17	26.992	133773	0.90	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-
18	27.034	187718	1.26	Hexadecane, 1,1-bis(dodecyloxy)-
19	27.100	286677	1.92	Hexasiloxane, tetradecamethyl-
20	27.175	336636	2.26	l-Methionine, N-benzyloxycarbonyl-, decyl ester
		14915335	100.00	



SI:89 Formula:C15H28O CAS:77899-117
MolWeight:224 RetIndex:1771 Comp
Name:(Z)6,(Z)9-Pentadecadien-1-ol (6Z,9Z)-6,9-
Pentadecadien-1-ol # $$ 100
90

Figure 3: Chemical structure of unsaturated alcohol determined by GC MS.

The laboratory experiment results showed significant differences in the attraction of winged males (Alate) to the pheromone extract (**Figure 5**) in a concentration treatment of 150 microliters, with an attraction rate of 41.67% (Table 2).

The results of Table 3 showed that the attraction of winged males was significantly higher after two days, reaching 33% compared to the one-day treatment and the control.

The results of FTIR spectroscopy analysis of the carrier materials (paraffin wax and cellulose fibers) revealed no forming effective chemical groups indicates to formation of a chemical reaction that may change the composition of the alcoholic extract of the pheromone substance , Rather, a process of physical mixing of the components occurred that helped cover the pheromone complex (coating) so that it could be released slowly into the environment. (Figure 6.)

Table 2: Termite alates that attract to pheromone extract at a different concentration under lab conditions.

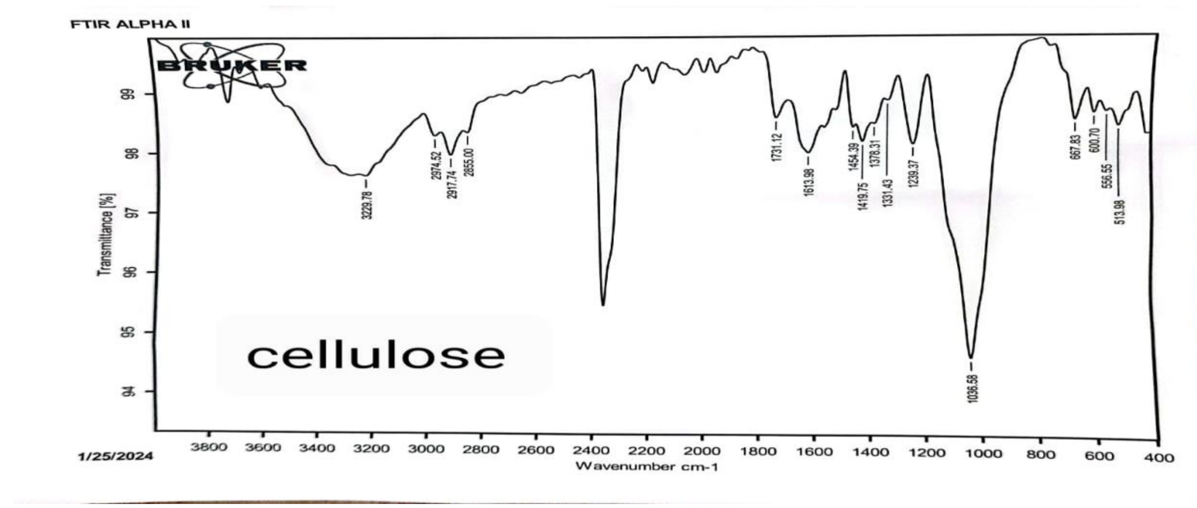
Concentrations	Percentage of Alates	Std. Deviation	Std. Error	sig
Control	0.0	0.0	0.0	c
50	13.3	10.3	4.2	b
100	23.3	10.3	4.2	b
150	41.6	9.8	4.0	a

Table 3: Termite alates that attract to pheromone extract at a different time under lab conditions.

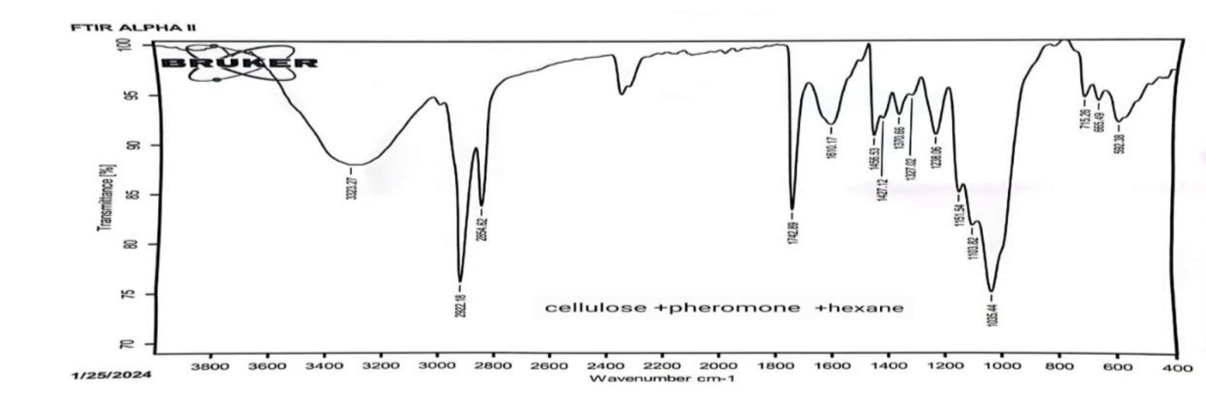
Time	Percentage of Alates	Std. Deviation	Std. Error	Sig
one day	20.0	9.2	3.2	b
two days	33.3	14.1	4.7	a
control	0.0	0.0	0.0	c

Figure 5: alates males that are attracted to pheromone extract at the laboratory.

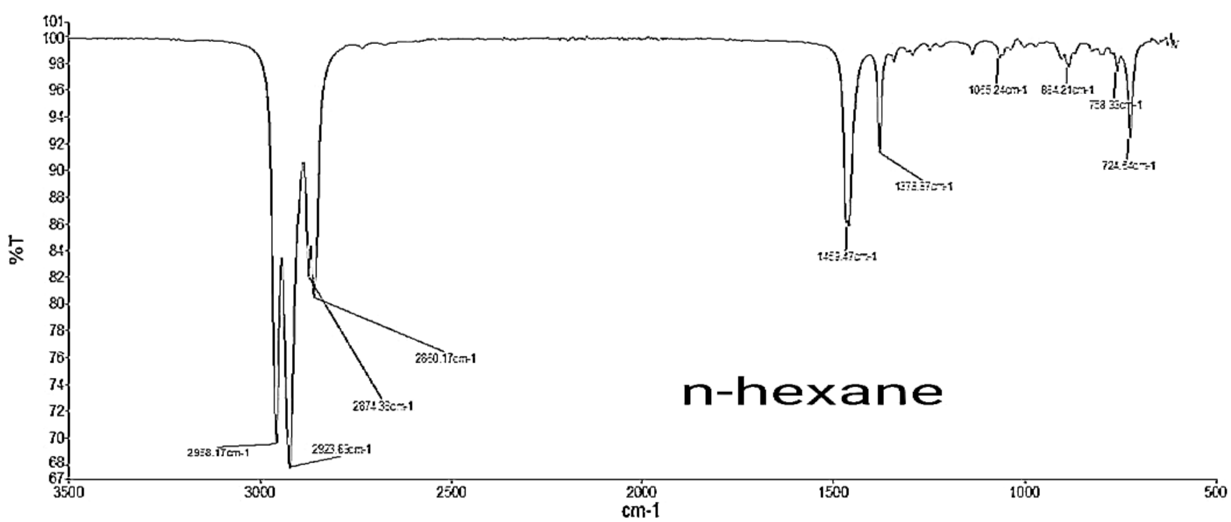
A


FTIR ALPHA II
BRUKER
Transmittance [%]
3229.78
2974.52
2917.74
2855.00
1731.12
1613.98
1454.39
1419.75
1378.31
1331.43
1239.37
1036.58
667.83
600.70
556.55
513.98
cellulose
1/25/2024
Wavenumber cm-1

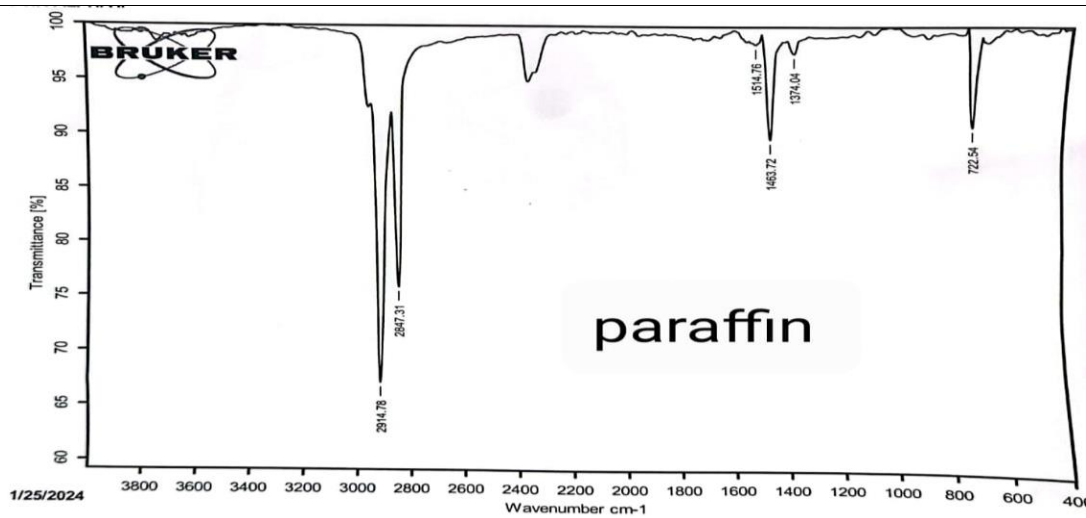
B


FTIR ALPHA II
BRUKER
Transmittance [%]
3323.27
2922.18
2854.62
1742.89
1610.17
1456.53
1427.12
1370.66
1327.02
1238.06
1161.54
1103.82
1035.44
715.25
665.49
592.38
cellulose +pheromone +hexane
1/25/2024
Wavenumber cm-1

C


%T
2958.17cm-1
2923.63cm-1
2874.38cm-1
2860.17cm-1
1459.47cm-1
1378.87cm-1
1065.24cm-1
894.21cm-1
758.33cm-1
724.54cm-1
n-hexane
cm-1

D



E

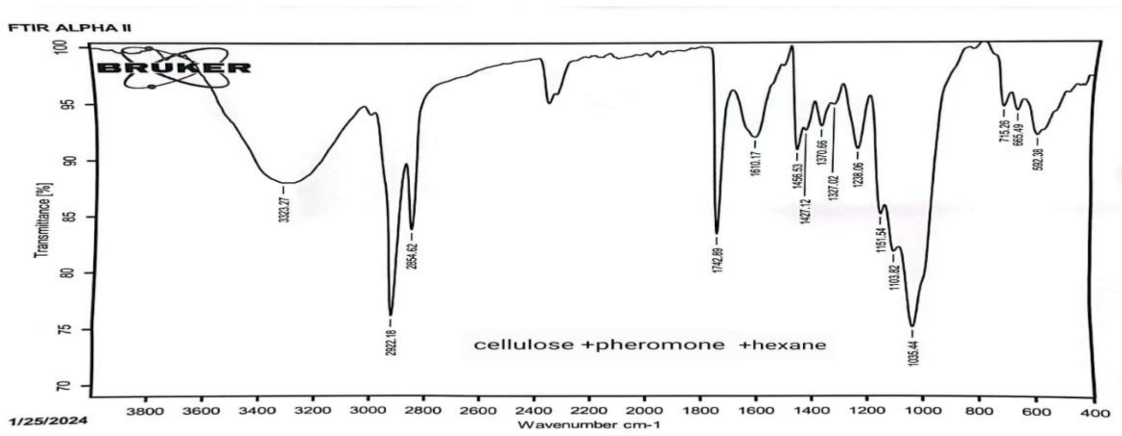


Figure 6: A: FTIR analysis of cellulose fibers (cellulose) B: FTIR analysis of the mixture of holocellulose and pheromone extract with n-hexane C: FTIR analysis of n-hexane D: FTIR analysis of paraffin E: FTIR analysis of the mixture of paraffin and pheromone extract with n-hexane**.**

The results of accumulated numbers of termite alates attracted to pheromone traps over time (Table 4) showed that highest attraction rate of attracting winged male termites occurred 7 days after the date of setting the traps in the orchard, as it reached 4.2 males/trap.

The results of the Effect of Polymer Carriers on Termite Alates Attracting to Pheromone Traps under Field Conditions (Table 5) revealed significant differences between the performance of various pheromone carrier formulations. The cellulose + pheromone trap achieved the highest attraction rate (8.667 males/trap), followed by the paraffin + pheromone trap (6.667 males/trap).

Table 4: Accumulative numbers of Termite alates that attract to pheromone traps at a different time under field conditions.

Days	Accumulative numbers of Termite alates	Std. Deviation	Std. Error	Sig
1	2.1	3.1	0.8	b
3	2.9	3.9	1.0	b
7	4.2	5.5	1.4	a

Table 5: Effect of Polymer Carriers on Termite alates attracting to pheromone traps under field conditions.

Trap type	Mean of Termite alates in traps	Std. Deviation	Std. Error	Sig
Control	0.0	0.0	0.0	c
cellulose only	0.0	0.0	0.0	c
paraffin only	0.0	0.0	0.0	c
cellulose+phromon	8.7	3.3	1.1	a
paraffin+pheromone	6.7	2.8	0.9	b

Termites are significant pests in palm orchards, especially their alate casts that swarm from the colonies during specific periods of the year, especially in the moderate temperatures of spring and fall (Abdulkader *et al*., 2011; Rad & Habibpour, 2018). Pheromone trapping is a behavioral control method that is environmentally safe. Few studies have been conducted on termite sex pheromones, due to the difficulty and complexity of these studies, which requires modern and complex chemical analysis devices. Bordereau *et al*.(2011) showed the sex-pairing pheromone, often secreted by the tergal glands of female alates, consists of a common compound (3Z,6Z,8E)-dodeca-3,6,8-trien-1-ol, a polyunsaturated alcohol. This compound is secreted by *Cornitermes cumulans*. However, in *C. silvestrii* , the pheromone includes (Z)-dodec-3-en-1-ol.

Dolejšová *et al*.(2018) studied the chemistry of sex-pairing pheromones and related mate-search behavior in winged (alates) of species: *Silvestritermes minutus*, *Embiratermes neotenicus*, and *S. heyeri*. Chemical analyses indicated that the females secrete specific combinations of unbranched, unsaturated C12 primary alcohols from the tergal glands. These include (3Z,6Z,8E)-dodeca-3,6,8-trien-1-ol and (3Z)-dodec-3-enol in *E. neotenicus*, (3Z,6Z)-dodeca-3,6-dien-1-ol in

S. heyeri , and(3Z,6Z)-dodeca-3,6-dien-1-ol and (3Z)-dodec-3- enol in *S.minutus*. These simple unsaturated alcohol compounds are consistent with those found in the abdominal tergite, and sternite alcoholic extract of *Microcerotermes diversus* Silvestri, specifically (Z)6, (Z)9-Pentadecadien-1-ol, as noted in the current study. Pheromone communication in insects is affected by many factors, including the concentration of the released pheromone and the time required for the insect needs to reach its target. Habib *et al*. (2018) found that increasing the concentration of pheromone in traps significantly enhanced the attraction of males of red palm weevil *Rhynchophorus ferrugineus*. As for Mihou et al.(2007) found that increasing the dose of dodecyl pheromone enhanced attracting males of the cornstalk borer *Sesameia* spp to the traps. Similarly, Wen et al.(2015) also showed that the termite *Ancistrotermes dimorphus* gave a response of attraction towards the female pheromone as the concentration increased from 0.1 nanogram to more than 1 nanogram under laboratory conditions. Cellulose, or dry wood tissue, is an important food item for termites, and using it as a carrier material may have greatly helped attract males ready to mate. Perhaps it is instinctive that it knows that females choose locations with available food sources (cellulose) for building their colonies. This could explain why cellulose pheromone traps are highly effective. Hellmann *et al*.(2011) successfully used cellulose amide fibers as a carrier of the insect pheromone, with the effectiveness of the pheromone release extending over more than three weeks in a slow-release process. It is worth noting that the traps set 7 days after the start of the experiment coincided with the peak of the termite Swarming, which was on the 4^{th} to 5^{th} of April in southern Iraq. This timing helped to give the best results for attraction; these findings were consistent with what Abdulkader *et al*., (2011) demonstrated in their study about the flight activity of alate termites in southern Iraq.

The study concluded that the unsaturated alcoholic compounds in the alcoholic extract, including the compound (Z)6, (Z)9-Pentadecadien-1-ol , effectively attracted winged males in the laboratory. The optimal amount for achieving the best results was 150 microliters, extracted from tergates and stearnites of 250 female termites. In the field experiment, pheromone traps that used cellulose as a carrier material were better than paraffin in attracting males. The alcoholic extract is composed interfering with many chemical compounds, Therefore, we recommend, if possible, purifying specific unsaturated alcohols to potentially achieve better results in the attraction process. Also, the alate males of the termites are not a good flying compared to the males of the insect species belonging to the order Lepidoptera and Hymenoptera, which indicates the presence of small means numbers attracted by the traps.

CONCLUSIONS

The crude alcoholic extract of sternum and tergite glands of termite , provided better indicators of the attraction of male winged termites (alates) to Jackson sticky traps, which were loaded onto cellulose compounds, compared to those loaded onto paraffin materials, both in the laboratory and orchard circumstances .

Conflict of Interest

The authors declare no conflict of interest associated with this manuscript.

Acknowledgment

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