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The Discovery of the Potential Attractive Compounds of *Bactrocera dorsalis* (Hendel)

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Abstract: *Bactrocera dorsalis* (Hendel) (*B. dorsalis*) is an important agricultural invasive pest that causes significant economic losses in tropical and subtropical fruit and vegetable crops. In this study, the proteins related to the sense of smell and taste of *B. dorsalis*, such as OBP, PBP, OR, IR, SNMP and CSP, were screened based on *B. dorsalis* transcriptome data. By integrating the compounds that were reported to be attractive to *B. dorsalis*, similar compounds of hydrocarbon compounds were obtained. Molecular docking was used to predict the binding between the similar compounds and the OBP, PBP, OR, IR, SNMP and CSP proteins. Network pharmacology was used to screen the potentially attractive compounds, and ecological experiments with *B. dorsalis* were finally conducted to verify the effect of these potentially attractive compounds on *B. dorsalis*. The results showed that the G protein-coupled receptor [BR: KO04030] and ion channel [BR: KO04040] pathways were closely related to the odor tropism of *B. dorsalis*. A total of 84 compounds, such as mitemcinal, exemestane and midecamycin, have potential binding effects on the *B. dorsalis* odor receptor proteins. The results of the ecological experiments showed that 1 mg/mL and 0.1 mg/mL 19-norandrostenedione, 1 mg/mL progesterone compounds was significantly attractive to *B. dorsalis* males, while 0.1 mg/mL exemestane was significantly attractive to *B. dorsalis* females. In this study, network pharmacology technology was used to discover the potential attractive compounds for *B. dorsalis*, which is important for the development and subsequent prevention and control of *B. dorsalis*. It can provide a reference in improving the success rates of clinical trials of new pest control products and in reducing the time and cost of drug development.

Keywords: *Bactrocera dorsalis* (Hendel); olfactory protein; network pharmacology; molecular docking; insect attractant

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1. Introduction

Bactrocera dorsalis (Hendel) (Diptera: Tephritidae), commonly known as the oriental fruit fly, is a highly destructive and invasive pest that poses a significant threat to agricultural crops in the Asia–Pacific region [1]. *B. dorsalis* has been listed among quarantine targets, and strict quarantine measures on fruit import and export have been implemented in many countries and regions [2]. It has developed various adaptive mechanisms that have

aided its successful establishment in both native and invasive habitats [3,4]. Due to the cryptic feeding habits of its larval stages and its pupation in soil, the management strategies of *B. dorsalis* are mainly focused on the control of adults [5]. Currently, olfaction-based adult trapping is one of the cost-effective tools in the control of *B. dorsalis* [6], such as the olfaction-based trapping agent methyl eugenol (ME), a naturally occurring compound in some plants [7]. ME has been widely used as a male attractant to monitor and control *B. dorsalis* populations for seven decades, used alone or in combination [7,8]. However, there is still room for the improvement of ME. For example, it was reported that the field populations of *B. dorsalis* had lower ME sensitivity compared to the susceptible strain [8]. Therefore, it is of great significance to develop a new environmentally friendly attractant for *B. dorsalis*.

Network pharmacology is an emerging technology that can extract the key information from the complex biological networks of gene, protein or compound interactions, thereby screening out the potential targets for therapeutic intervention [9,10]. Network pharmacology has been successfully used to demonstrate the complex mechanisms of TCM in treating diseases based on multi-compounds, multi-targets and multi-pathways [9,11]. It has been used extensively in medical drug development to improve the success rate of clinical trials for new drugs and to decrease the cost of drug development, but it has been used less for drug development on insects.

The perception of odor in insects is a multifaceted process that encompasses various proteins, including olfactory receptors (ORs), odorant-binding proteins (OBPs), gustatory receptors (GRs), ionotropic receptors (IRs), and sensory neuron membrane proteins (SNMPs) [12–15]. ORs constitute a diverse family of membrane protein receptors that are responsible for the majority of insect olfactory perception and communication. Therefore, these various proteins are crucial for the development of repellents or pesticides [15]. Several proteins associated with odor recognition have been identified in *B. dorsalis* [16]. For instance, the ORs BdorOR94b-2/Bdor ORCO exhibited a response to isoeugenol, while BminOBP9 could potentially identify citrus volatiles specifically [17]. The primary current research approach is to identify a suitable priming substance and study its related proteins at the molecular level [17,18].

These methods provide a theoretical foundation for basic research on *B. dorsalis*. On this basis, the use of network pharmacology, transcriptomics and current database data (such as NCBI) can provide faster methods for the discovery and identification of various odor-sensitive proteins and the development of attractants in *B. dorsalis*.

In this study, a number of proteins associated with *B. dorsalis* olfaction, including OBP, pheromone-binding protein (PBP), OR, IR, SNMP and chemosensory proteins (CSPs), were identified by screening the *B. dorsalis* transcriptome. We systematically gathered and analyzed compounds that were reported to have an attractive effect on *B. dorsalis*. Molecular docking, network pharmacology and ecological experimentation were combined together and used in this study. In order to predict the potential attractive compounds, we use molecular docking and network pharmacology technology. The predicted potential attractive compounds were verified by the behavior of *B. dorsalis*. This study presents a novel method for effectively screening potential attractants for *B. dorsalis*. Additionally, this study introduces new insights into the role of odor proteins and providing a valuable reference for the development of attractants for *B. dorsalis*.

2. Materials and Methods

2.1. Insect Rearing

B. dorsalis was collected from the Hainan University Fruit Tree Plantation (Haikou, China) and reared as previously described [19] in the Invasive Pest Laboratory, Hainan University (Haikou, China). The larvae of the *B. dorsalis* colony were provided an artificial larval diet mixture of 50 g torula yeast, 250 g wheat power bran, 50 g sugar, 1 g sodium benzoate, 50 g scraps of paper and 400 mL water. Adult flies were fed artificial diets of 3:1 sucrose:yeast extract. All the experimental insects were maintained in cages

(60 × 60 × 60 cm) at 27 ± 1 °C under a 16 h/8 h light/dark cycle at a relative humidity of 70% ± 5%.

2.2. Collection, Identification and Enrichment of Olfactory Sensory Proteins in B. dorsalis

2.2.1. RNA Extraction, cDNA Library Preparation and Sequencing

Total RNA was isolated from the following developmental stages: adult chemosensory tissues, including antennae, mouthparts, thoracic leg and female ovipositor (within six days of eclosion) in a 1:1 female:male ratio. All samples were snap-frozen in liquid nitrogen and stored at −80 °C until total RNA was extracted. Samples were sent to Gene Denovo (Guangzhou, China). Construction of normalized cDNA libraries from the 14 *B. dorsalis* samples and 454 pyrosequencing were carried out as follows. First, total RNA was extracted from each sample using TRIzol reagent (Life Science Technologies-Invitrogen, Carlsbad, CA, USA), and the quantity and quality of RNA were assessed by spectrophotometry and gel electrophoresis. Then, mRNA was isolated from 20 µg of each total RNA using the Oligotex mRNA Mini Kit (Qiagen, Valencia, CA, USA). First-strand cDNA was synthetized from 1 µg mRNA with a Super Script III reverse transcriptase using a dT15VN2 primer (Invitrogen) under the following conditions: 5 min at 65 °C, 2 min at 4 °C, 1 h at 42 °C and 10 min at 70 °C in a PCR machine (Bio-Rad, Hercules, CA, USA). The second strand was synthesized from 1 µL of the first-strand cDNA reaction mix using DNA ligase, DNA polymerase I and RNase H from *E. coli* according to the manufacturer's instructions (Invitrogen). T4 DNA polymerase was added and incubated for 5 min at 16 °C in a PCR machine. The synthesized double-stranded cDNA was purified with the QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA), and the yield was determined using a TBS 380 fluorometer (Turner Biosystems, Sunnyvale, CA, USA). Subsequently, cDNA was fragmented by sonication and the cDNA samples ranging in size from 100 bp to 800 bp were purified on 2% agarose gel. Then, DNA concentration in each cDNA sample was determined using the Bioanalyzer DNA1000 Kit (Agilent, Santa Clara, CA, USA). Each purified cDNA sample was then used to synthesize single-strand template DNA (sstDNA) libraries using the GS20 DNA Library Preparation Kit (Roche Applied Science, Penzberg, Germany) following the manufacturer's recommendations (1/4 run for each sample). Library quality was assessed on an Agilent Bioanalyzer High Sensitivity DNA chip. Finally, each library was normalized in equimolar concentrations and diluted to 1 × 106 molecules/µL. Emulsion-based clonal amplification and sequencing were performed on the 454 Genome Sequencer FLX Titanium System according to the manufacturer's instructions (454 Life Sciences, Branford, CT, USA).

2.2.2. Gene Annotation and Sequence Analysis

Amino acid sequences predicted from the assembled 454 sequences were compared to protein sequences in the NCBI non-redundant (nr) protein database on a local server using the BLASTALL program with the cutoff e-value of 10^{-5} [20]. GO annotation was performed using Blast2 GO. GO association was performed by BLASTX comparison against the NCBI nr database [21,22]. To specifically annotate the OBPs, CSPs, ORs, IRs, GRs and SNMPs in *B. dorsalis*, assembled sequences were analyzed using TBLASTN and TBLASTX programs against custom-made databases consisting of insect sequences processed using the BioEdit program [23]. The sequences whose best TBLASTN hits corresponded to OBPs, CSPs, ORs, IRs, GRs and SNMPs were then retained as candidate *B. dorsalis* chemosensory transcripts, and their translation was manually verified and corrected if needed. Finally, families of all candidate *B. dorsalis* chemosensory protein sequences were analyzed in Pfam. Then, open reading frames (ORFs) in the assembled full-length UniGenes were identified using the ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html, accessed on 15 November 2023). The signal peptides of OBPs and CSPs were predicted using SignalP 4.0 [24]. Transmembrane domains of candidate ORs, IRs, GRs and SNMPs were predicted using TMHMM 2.0 [25]. The deduced protein sequences were further confirmed by searching the Pfam database with default parameters and an e-value of 1.0. Based on

these searches, putative chemosensory genes in the *B. dorsalis* transcriptome were named after their *Drosophila* homologues.

2.2.3. Phylogenetic Analyses

Phylogenetic analyses of the *B. dorsalis* chemosensory genes were constructed based on the amino sequences after the removal of the signal peptides and the data set was collected from NCBI. The phylogenetic tree is based on the genes obtained from the transcriptome and the gene families of the Supplementary Table S1s in the genome above NCBI [26]. The msa package of R software (version 4.3.1) was used in the sequence alignment, the ape package and the neighbor joining algorithm were used in the construction of phylogenetic trees, and the ggtree package was used in the construction of the graphs.

The OBPGOBP data set contained 340 sequences from *Bactrocera cucurbitae* (*B. cucurbitae*), *B. dorsalis*, *Bactrocera oleae*, etc. [27,28]. The CSP data set contained 58 sequences from *Acyrthosiphon pisum*, *Bombyx mori*, etc. The OR data set contained 690 sequences from *B. cucurbitae*, *B. dorsalis*, etc. The IR data sets contained 223 sequences from *B. cucurbitae*, *B. dorsalis*, etc. The SNMP data sets contained 100 sequences from *B. cucurbitae*, *B. dorsalis*, etc. The PDB data sets contained 27 sequences from *B. cucurbitae*, *B. dorsalis*, etc. (Supplementary Table S1).

2.2.4. GO Functional Enrichment and KEGG Pathway Enrichment Analyses

GO enrichment analysis was performed on the targets of olfactory sensory proteins. Using the String database, terms with a corrected value < 0.05 were selected. Next, the Cluster Profiler package of R 4.3.1 software was adopted to conduct GO enrichment [29]. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of core targets was also carried out and visualized using the ggplot2 R package. A p-value < 0.05 was set to be significant. A suite of KEGG mapping tools, KEGG Mapper, was used for specific pathway visualization [30].

2.3. Collection of Potential Attractive Compounds

A total of 115 compounds that were related to *B. dorsalis* attraction were collected from published reports. These 115 compounds were classified based on the public database PubChem (https://pubchem.ncbi.nlm.nih.gov/, accessed on 15 November 2023) (Supplementary Table S2). Hydrocarbons contained the most compounds of any classification, with 39 found. A total of 86 analogues were used to collect the top 5 analogues of Score in the public database Swiss Similarity (http://www.swisssimilarity.ch/, accessed on 15 November 2023) using hydrocarbons (Supplementary Table S3). The 3D and 2D structures of the analogues were collected from the PubChem database and were used in the molecular docking. The protein structures of the classified *B. dorsalis* genes of OBP, PBP, OR, IR, CSP and SNMP were built based on the public database Swiss Model (https://swissmodel.expasy.org/interactive, accessed on 15 November 2023).

2.4. Molecular Docking

SDF formats of 2D or 3D structures of 86 analogue molecules were converted to MOL2 format with Open Babel 3.1.1 software. The docking of the 86 compounds and the protein structures of *B. dorsalis* were conducted with AutoDock Vina software (version 1.2.3). Based on the molecular docking, the effectiveness of the interactions between proteins and compounds could be evaluated by the binding energy with the evaluation criterion of a lower value of affinity representing a better binding energy [31,32]. The binding ability between proteins and compounds was judged with the affinity value, while the value equal to or less than −5 kcal/mol and greater than or equal to −18 kcal/mol was considered to be the effective binding value. Visual diagrams were created using PyMOL 2.5.4 software.

2.5. Screening and Prediction of Core Functional Compounds

Based on molecular docking, the binding relationships between *B. dorsalis* olfactory sensory-related proteins and similar compounds of potential attraction were constructed. Enrichments of the *B. dorsalis* taste sensation-related proteins were conducted. After these enrichments, the relationships between these proteins and the pathways from the enrichments were construed and obtained. A composite network of compounds vs. proteins vs. pathways vs. GO terms was constructed. In this network, the importance of some nodes came from their own weights and the interactions between adjacent nodes. We used degrees to represent the importance of a compound in this network, with a greater degree representing a more important role a node played in the network, meaning that the node was the more important factor in the relationships among the comprehensive effects among genes, compounds, KEGG pathways and GO terms. The compounds with a higher degree were regarded as having the most attractive effects on *B. dorsalis*. The top 2 KEGG pathways, 51 GO terms and the olfactory sensory proteins were imported into the Cytoscape 3.9.1 software to construct the compound–pathway–GO term–olfactory sensory protein network (CPGP network) [33].

2.6. Behavioral Assays

Behavior assays were conducted in an independent behavioral assay laboratory. The behavioral assay laboratory was fitted with exhaust fans for ventilation and maintained at a 25 ± 2 °C temperature, 70% ± 5% RH and 16 h:8 h, light:dark photoperiod. Twenty-five cages were placed inside the behavioral assay laboratory (each L30 cm × W30 cm × H30 cm). A water box and a white lure bottle were placed in each cage. The water box was used to provide water for *B. dorsalis*, and the attracting bottle was used to contain attracting substances to attract *B. dorsalis*. In order to reduce the potential for interference between each treatment as much as possible, each potential attractant test was conducted in an independent room and five replicates were conducted at a time.

A total of six treatments were conducted. Treatments 1–3 are the treatment groups. Treatments 4–6 are the control groups.

Treatment 1: Attractiveness of exemestane to *B. dorsalis*. Exemestane was dissolved in 5 μL dimethyl sulfoxide (DMSO, RT, 99%) and then distilled water was added. At the same time, according to existing reports, the concentration of DMSO used in cell experiments should be less than 0.1%, and so the concentration of DMSO used in this study was less than 0.1% [34]. Exemestane was prepared into 1 mg/mL, 0.1 mg/mL and 0.01 mg/mL solution. A total of 200 μL of 1 mg/mL, 0.1 mg/mL and 0.01 mg/mL solution was added to the lure bottle in the cage. At the same time, we put 50 adults of *B. dorsalis* into a cage (male:female 1:1; 10 days old, starved for 24 h). All treatments were carried out 1 h after the start of illumination, and the number of male- and female-attracted *B. dorsalis* in the bottle was calculated after 24 h.

Treatment 2: Attractiveness of progesterone to *B. dorsalis*. The same experimental protocol was adopted as in Treatment 1 except that exemestane was replaced with progesterone.

Treatment 3: Attractiveness of 19-norandrostenedione to *B. dorsalis*. The same experimental protocol was adopted as in Treatment 1 except that exemestane was replaced with 19-norandrostenedione.

Treatment 4: Attractiveness of DMSO to *B. dorsalis*. The same experimental protocol was adopted as in Treatment 1 except that exemestane was replaced with 200 μL solvent (0 mg/mL; solvent made of 5 μL DMSO and distilled water, no potential attractant substances added).

Treatment 5: Attractiveness of empty bottle to *B. dorsalis*. The same experimental protocol was adopted as in Treatment 1 except that the lure bottle was empty.

Treatment 6: Attractiveness of ME to *B. dorsalis*. The same experimental protocol was adopted as in Treatment 1 except that that exemestane was replaced with 1 mg/mL ME (ME was dissolved in 5 μL DMSO and distilled water to prepare a solution with a concentration of 1 mg/mL).

Data analysis was conducted by Poisson distribution of a non-normal distribution in a generalized linear model. Then, analysis of variance and multiple comparisons were used for significance analysis. Finally, a histogram was created using the ggplot2 package. The data were analyzed with packages multcomp, glrm, emmeans, lsmeans, ggsignif and ggplot2 in R 4.3.1 software.

$$\text{Male proportion} = \frac{\text{Attracted males}}{\text{Attracted males} + \text{Attracted females}} \times 100\%$$

$$\text{Adult attraction rate} = \frac{\text{Attracted males} + \text{Attracted females}}{50} \times 100\%$$

$$\text{Male attraction rate} = \frac{\text{Attracted males}}{25} \times 100\%;\ \text{Females attraction rate} = \frac{\text{Attracted females}}{25} \times 100\%.$$

3. Results

3.1. Collection, Identification and Enrichment of Olfactory Sensory Proteins in B. dorsalis

The transcriptome sequencing of 14 samples was completed, and a total of 77.52 Gb of clean data were obtained, with the clean data of each sample reaching 4.04 Gb and the percentage of Q30 bases being 96.11% and above. A total of 77,765 UniGenes were obtained after assembly, of which 20,212 UniGenes were over 1 kb. Functional annotation of UniGenes (comparison of NR, Swiss-Prot, KEGG, COG, KOG, GO and Pfam databases) was performed, and a total of 44,123 UniGene annotation results were obtained. Gene structure analysis was performed based on UniGene libraries, in which SSR analysis yielded a total of 10,334 SSR markers.

3.1.1. Phylogenetic Analyses

To assign putative functions to olfactory protein genes, we determined the phylogenetic relationships between the 24 IRs identified in this study, and the 223 IRs previously reported in *Dmel* and other tephritid species. As expected, the *B. dorsalis* IRs clustered together with orthologous IRs from *Dmel* and other tephritids with the best BLASTP hit. The 27 *B. dorsalis* IRs were distributed in three well-distinct clades together with homologous genes from tephritid species. The *c59342.graph_c0*, which had robust expression levels in the antennae, also clustered together with BdorIR25a (Supplementary Figure S1F).

Furtherly, the phylogenies of two CSP genes, twenty-eight OBPGOBP genes, fifty-seven OR genes, one PBP gene and four SNMP genes were identified (Supplementary Figure S1A–F). A total of 116 genes were identified as being related to olfactory protein genes (Supplementary Table S1).

3.1.2. GO Functional Enrichment and KEGG Pathway Enrichment Analyses

The IR, OBP, OR, PBP, SNMP and CSP genes were identified from the transcriptome and phylogenetic analyses, and they were taken into the enrichments of GO and KEGG. Two KEGG pathways were obtained and are shown in a bar graph (Figure 1A), and the top 20 GO terms measured with the number of genes are shown in a bubble graph (Figure 1B). The results showed that this group of genes related to *B. dorsalis*'s odor were mainly concentrated in two pathways, the G protein-coupled receptor pathway [BR:ko04030] and ion channel pathway [BR:ko04040], with 30 and 10 genes, respectively. Among them, sensory perception of smell, detection of chemical stimuli and detection of stimuli involved in sensory perception GO terms were enriched with more genes. Among these pathways and GO terms, several lines of recent evidence indicate that ion channels play a key role in cellular signaling and tissue morphogenesis, and ion channels have been found to play a key role in the early developments of *Drosophila melanogaster* [35,36], indicating that these IR, OBP, OR, PBP, SNMP and CSP genes may play important roles in *B. dorsalis*.

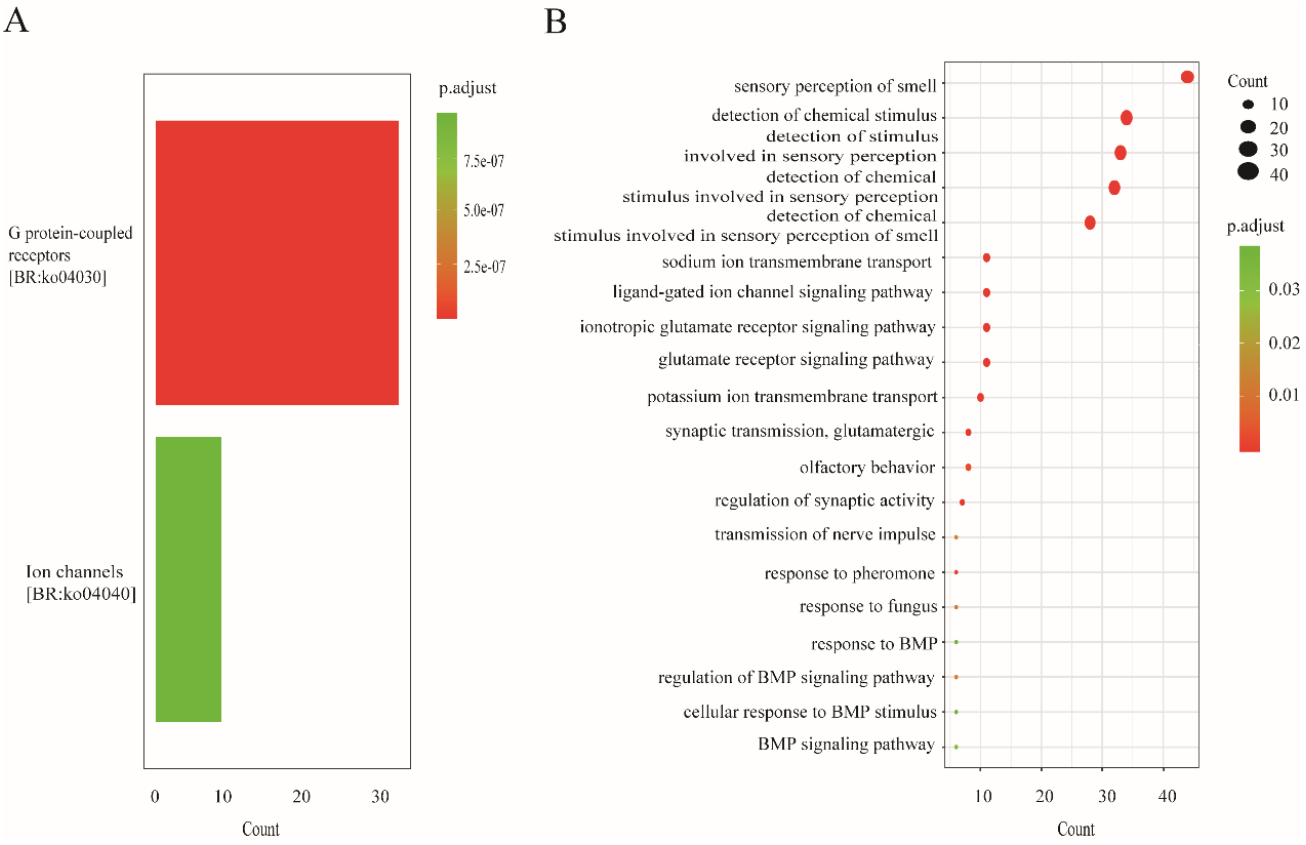

Figure 1. KEGG pathway and GO enrichment analyses of olfactory sensory genes. (**A**) KEGG pathway enrichments; (**B**) GO enrichments.

3.2. Collection of Potential Attractive Compounds

Based on the existing studies, we gathered 115 compounds that were reported to have attractive properties to *B. dorsalis*. The majority of these compounds were identified as hydrocarbons (Supplementary Table S2). After the analysis of the similar compounds of the 39 hydrocarbon compounds, we obtained a total of 86 compounds (Table 1; Supplementary Table S3). These 86 compounds were subsequently used for further analysis, screening and experiments.

Table 1. The 86 similar compounds of the 39 hydrocarbon compounds.

ID	English Name	CAS	ID	English Name	CAS
T01	(4R)-limonene	5989-27-5	T44	Pentolinium	144-44-5
T02	Toluene	108-88-3	T45	Ginkgolide-J	107438-79-9
T03	1,3,5-Trimethoxybenzene	621-23-8	T46	Decamethonium	156-74-1
T04	Chamazulene	529-05-5	T47	Isoquinoline	119-65-3
T05	Alpha-Pinene	80-56-8	T48	Cinnamyl alcohol	4407-36-7
T06	Anhydrovitamin A	1224-78-8	T49	Enbucrilate	6606-65-1
T07	Calcium undecylenate	1322-14-1	T50	Caprylyl glycol	1117-86-8
T08	Farnesol	4602-84-0	T51	Carbazole	86-74-8
T09	Spermine	71-44-3	T52	Prifinium	10236-81-4
T10	Androstenedione	63-05-8	T53	Lauric acid	143-07-7

Table 1. *Cont.*

ID	English Name	CAS	ID	English Name	CAS
T11	Terpineol	8000-41-7	T54	MDL72527	99207-33-7
T12	Adamantane	281-23-2	T55	17alpha-methyl-3beta	571-03-9
T13	Verbenone	80-57-9	T56	Boldione	897-06-3
T14	Mitemcinal	154738-42-8	T57	Hexamethonium	60-26-4
T15	Dodecyltrimethylammonium	10182-91-9	T58	Ginkgolide-C	15291-76-6
T16	Anethole	4180-23-8	T59	Cetyltrimethylammonium naproxenate	102580-74-5
T17	Levoverbenone	1196-01-6	T60	Benzyl formate	104-57-4
T18	19-norandrostenedione	734-32-7	T61	Hydroxytyrosol	10597-60-1
T19	Terpinyl acetate	8007-35-0	T62	Amyl acetate	628-63-7
T20	Nonan-1-Ol	28473-21-4	T63	Tetraethylammonium	66-40-0
T21	Beta-Pinene	127-91-3	T64	Capric acid	334-48-5
T22	Bretylium	59-41-6	T65	Octamethylenediamine	373-44-4
T23	Soneclosan	3380-30-1	T66	4-Androstenediol	1156-92-9
T24	Guaiazulen	489-84-9	T67	5-androstenedione	571-36-8
T25	Undecylenic acid	112-38-9	T68	(S)-oct-1-en-3-ol	24587-53-9
T26	Levomenol	23089-26-1	T69	Dimethyl carbate	39589-98-5
T27	Spermidine	124-20-9	T70	Ginkgolide-M	15291-78-8
T28	Geraniol	106-24-1	T71	Quaternium-24	32426-11-2
T29	Terpinen-4-ol	562-74-3	T72	Exemestane	107868-30-4
T30	Camphane	464-15-3	T73	Bornyl acetate	76-49-3
T31	Fusicoccin	20108-30-9	T74	17beta-diol	1852-53-5
T32	Dioctyldimonium	20256-55-7	T75	Iodobenzene	591-50-4
T33	Vanillyl alcohol	498-00-0	T76	Carbaryl	63-25-2
T34	2-octyl cyanoacrylate	133978-15-1	T77	N-Tridecanoic Acid	638-53-9
T35	1-Dodecanol	112-53-8	T78	Agmatine	306-60-5
T36	1,2-dichlorobenzene	95-50-1	T79	Bolandiol	19793-20-5
T37	Triclosan	3380-34-5	T80	Atamestane	96301-34-7
T38	Diphemanil	15394-62-4	T81	Dibromothymoquinone	29096-93-3
T39	Palmitoleic Acid	373-49-9	T82	Midecamycin	35457-80-8
T40	Perillyl alcohol	18457-55-1	T83	Cetrimonium	6899-10-1
T41	Bis(6-aminohexyl)amine	143-23-7	T84	Benzyl benzoate	120-51-4
T42	Progesterone	57-83-0	T85	CA4P	222030-63-9
T43	Duroquinone	527-17-3	T86	Isopentyl 2-cyanoacrylate	19475-26-4

Note: CAS, Chemical Abstracts Service; ID, short names of the compounds in this study.

3.3. Molecular Docking

For selecting potential attractive compounds that could be effectively used in the behavioral assays, we conducted molecular dockings to 86 potential attractive compounds and the 116 genes that responded to the IR, OBP, OR, PBP, SNMP and CSP genes. A total of 246,558 binding relationships were obtained from the dockings (Supplementary Table S4).

The information on the binding relationships divided by the binding affinity values showed that 82 potential attractive compounds of the 86 potential attractive compounds could bind the 59 genes of the 116 genes with the effective binding affinities, equal or

lower than −5.00 kcal/mol. It was indicated that the 86 potential attractive compounds could well target the proteins of IRs, OBPs, ORs, PBPs, SNMPs and CSPs of *B. dorsalis* (Figure 2A,B).

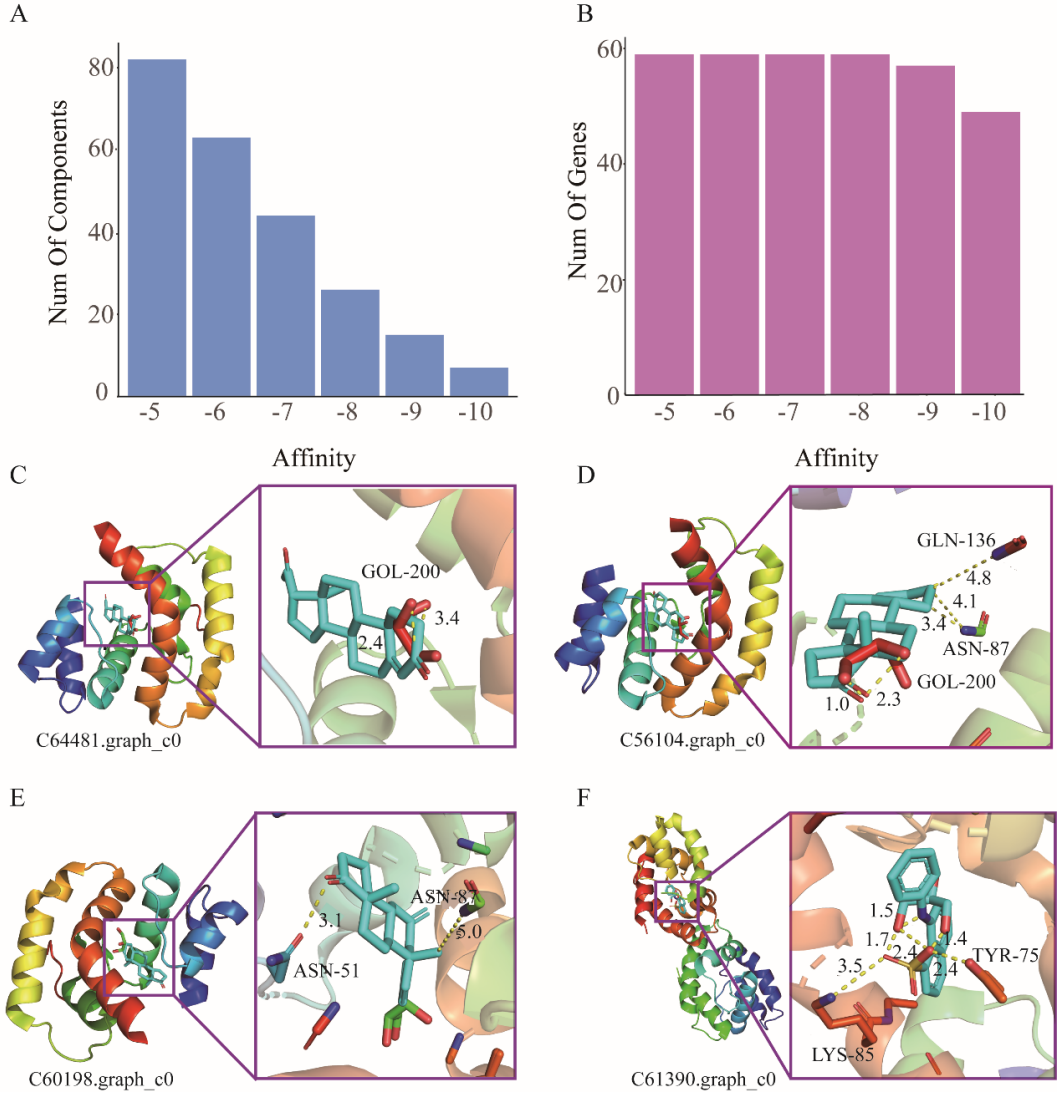

Figure 2. Visualization of four olfactory receptor proteins docking with potential attractive compounds. (**A**) The numbers of compounds with different affinities between −5 kcal/mol and −18 kcal/mol were counted. (**B**) The numbers of genes with different affinities between −5 kcal/mol and −18 kcal/mol were counted. (**C**) Gene *C64481.graph_c0* was identified as OR, docked from compound T42; (**D**) Gene *C56104.graph_c0* was identified as OBPGOBP, docked from compound T18; (**E**) Gene *C60198.graph_c0* was identified as OR, docked from compound T72; (**F**) Gene *C61390.graph_c0* was identified as OR, docked from compound T82.

Among the 246,558 binding relationships, the compound T42 could bind the best with the protein structure *c64481c04* of gene *C64481.graph_c0*, with a binding affinity of −9.42 kcal/mol (Figure 2C), followed by the binding relationships of T18 (*C56104.graph_c0*, −9.61 kcal/mol), T72 (*C60198.graph_c0*, −10.17 kcal/mol) and T82 (*C61390.graph_c0*, −9.50 kcal/mol) (Figure 2D,E). The average affinities of these four compounds were −6.38, −6.44, −6.59 and −6.69 kcal/mol, respectively.

3.4. Screening and Prediction of Core Functional Compounds

In the CPGP network, we constructed 144 common targets (Figure 3). A total of 5809 edges and 244 nodes were established in the network, representing node interactions (Supplementary Table S5). The sizes of the nodes were plotted based on the degree value, with a bigger node indicating a greater degree. The value of the degree represented the importance of the node in the network, while a greater value represented greater importance. The average degree of the CPGP network was 45.97. Topological analysis of the CPGP networks identified 61 genes and 51 compounds whose scores were greater than the value of 50 and were treated as the core targets. The compounds of T14, T70, T82, T72, T56, T55, T67, T18, T42 and T80 had greater degrees (Supplementary Table S4), meaning that these compounds play the most important roles in the network and may be the most important and potential attractive compounds for *B. dorsalis*. Among them, the node corresponding to the gene *c56889.graph_c0* had the highest degree and greatest interaction with each compound.

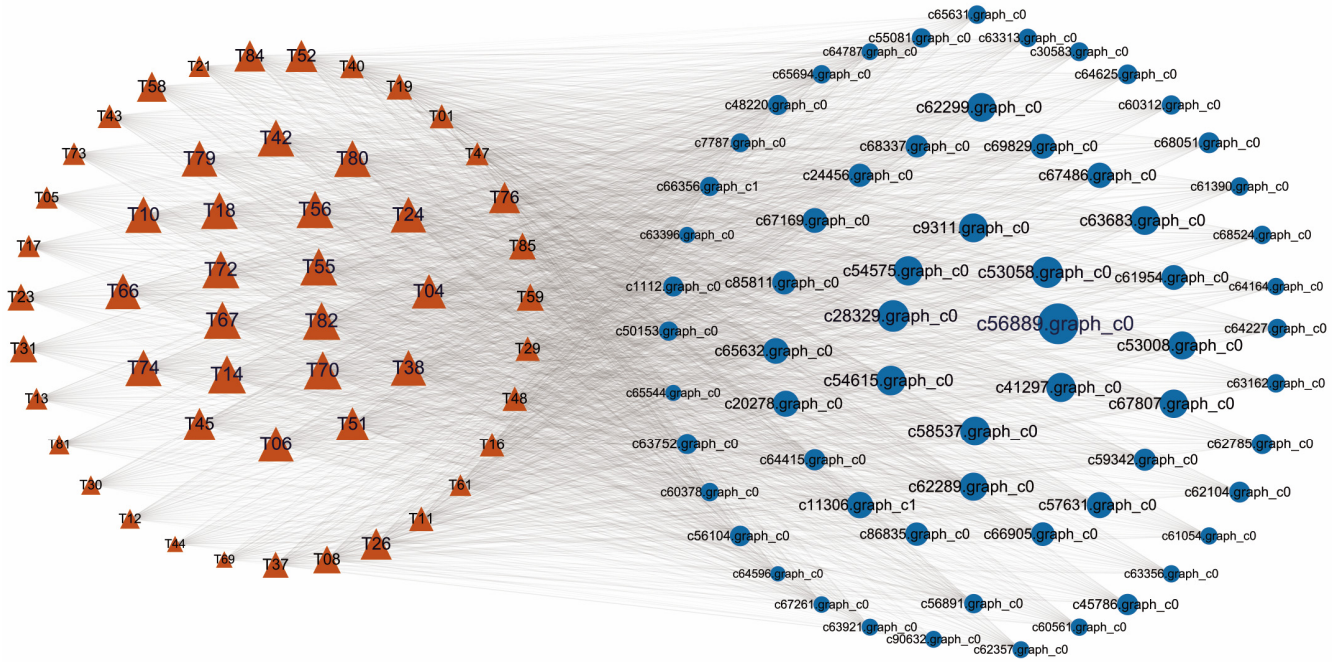

Figure 3. Network node diagram with corresponding potential attractive compounds and genes. The potential attractive compounds and identified olfactory receptor protein genes are highlighted in orange and blue, respectively.

3.5. Behavioral Assays

Based on the prediction of docking and core function, we selected the top 20 potentially attractive compounds based on their average affinity and degree (Table 2). As well as considering the economic applicability of the compounds, we selected three compounds, T72 (exemestane), T42 (progesterone) and T18 (19-norandrostenedione), to validate their elicitation ability against *B. dorsalis*.

The experiments and some reports showed that DMSO could be used as the solvent of compounds in the attraction experiments of *B. dorsalis*, while the number of *B. dorsalis* was not significant between the treatments with DMSO and empty bottles (Figure 4).

An amount of 0.1 mg/mL exemestane achieved a significantly higher adult attraction rate than DMSO and the empty bottle. But, ME achieved a significantly higher adult attraction rate than 0.1 mg/mL exemestane (Figure 4A). For the promotion of males, 0.1 mg/mL exemestane had an attraction rate of 28.20% to males (Table 3), so it was deemed not attractive to males. An amount of 0.1 mg/mL exemestane achieved a significantly higher female attraction rate than DMSO and the empty bottle (Figure 4B).

Table 2. The top 20 potentially attractive compounds of average affinity and degree.

Compound ID	Compound Name	CAS	Average Affinity (kcal/mol)	Degree
T70	Ginkgolide-M	15291-78-8	−10.12	107
T14	Mitemcinal	154738-42-8	−8.77	107
T82	Midecamycin	35457-80-8	−6.69	106
T72	Exemestane	107868-30-4	−6.59	105
T55	17alpha-methyl-3beta	571-03-9	−6.55	105
T56	Boldione	897-06-3	−6.50	105
T67	5-androstenedione	571-36-8	−6.42	105
T42	Progesterone	57-83-0	−6.38	104
T80	Atamestane	96301-34-7	−6.51	104
T18	19-norandrostenedione	734-32-7	−6.44	104
T74	17beta-diol	1852-53-5	−6.52	103
T10	Androstenedione	63-05-8	−6.51	103
T79	Bolandiol	19793-20-5	−6.49	102
T06	Anhydrovitamin A	1224-78-8	−6.25	101
T66	4-Androstenediol	1156-92-9	−6.48	101
T38	Diphemanil	15394-62-4	−6.20	101
T24	Guaiazulen	489-84-9	−6.39	101
T04	Chamazulene	529-05-5	−6.24	98
T51	Carbazole	86-74-8	−6.18	96
T52	Prifinium	10236-81-4	−6.06	92

Table 3. The numbers of different compounds attracted by *B. dorsalis* and the proportions of males in the attracted adults.

Concentration	Number of *B. dorsalis* Mean ± SE	Male Proportion
1 mg/mL Exemestane	3.60 ± 0.49 ^{ab}	22.22%
0.1 mg/mL Exemestane	7.80 ± 0.98 ^{bc}	28.20%
0.01 mg/mL Exemestane	4.60 ± 0.80 ^{ab}	24.78%
1 mg/mL Progesterone	11.00 ± 1.26 ^{c}	70.90%
0.1 mg/mL Progesterone	3.60 ± 0.49 ^{ab}	55.55%
0.01 mg/mL Progesterone	3.40 ± 0.49 ^{ab}	58.82%
1 mg/mL 19-norandrostenedione	8.00 ± 0.40 ^{bc}	95.00%
0.1 mg/mL 19-norandrostenedione	8.60 ± 1.01 ^{bc}	93.02%
0.01 mg/mL 19-norandrostenedione	2.40 ± 0.49 ^{a}	100.00%
DMSO Solvent (0 mg/mL)	4.40 ± 0.49 ^{ab}	45.00%
Empty bottle	2.20 ± 0.40 ^{a}	45.00%
1 mg/mL ME	11.40 ± 1.01 ^{c}	85.96%

Note: Values with the same small letters in the same column are not significantly different at 0.05 levels. Statistical significance was determined using multiple comparisons.

An amount of 1.00 mg/mL progesterone achieved a significantly higher adult attraction rate than DMSO and empty bottle. But, ME achieved a significantly higher adult attraction rate than 1.00 mg/mL progesterone. For the promotion of males, 1.00 mg/mL progesterone had an attraction rate of 70.90% to males, so it was deemed attractive to males. It achieved a significantly higher male attraction rate than DMSO and the empty bottle, but it was under the value for ME (Figure 4, Table 3).

An amount of 1.00 mg/mL and 0.10 mg/mL 19-norandrostenedione achieved a significantly higher adult attraction rate than the empty bottle and was deemed attractive to males. It achieved a significantly higher male attraction rate than DMSO and the empty bottle, but was under the value for ME. For the promotion of males, 1.00 mg/mL and 0.10 mg/mL 19-norandrostenedione had attraction rates of 95.0% and 93.0% to males, respectively, but they were not attractive to females (Figure 4, Table 3).

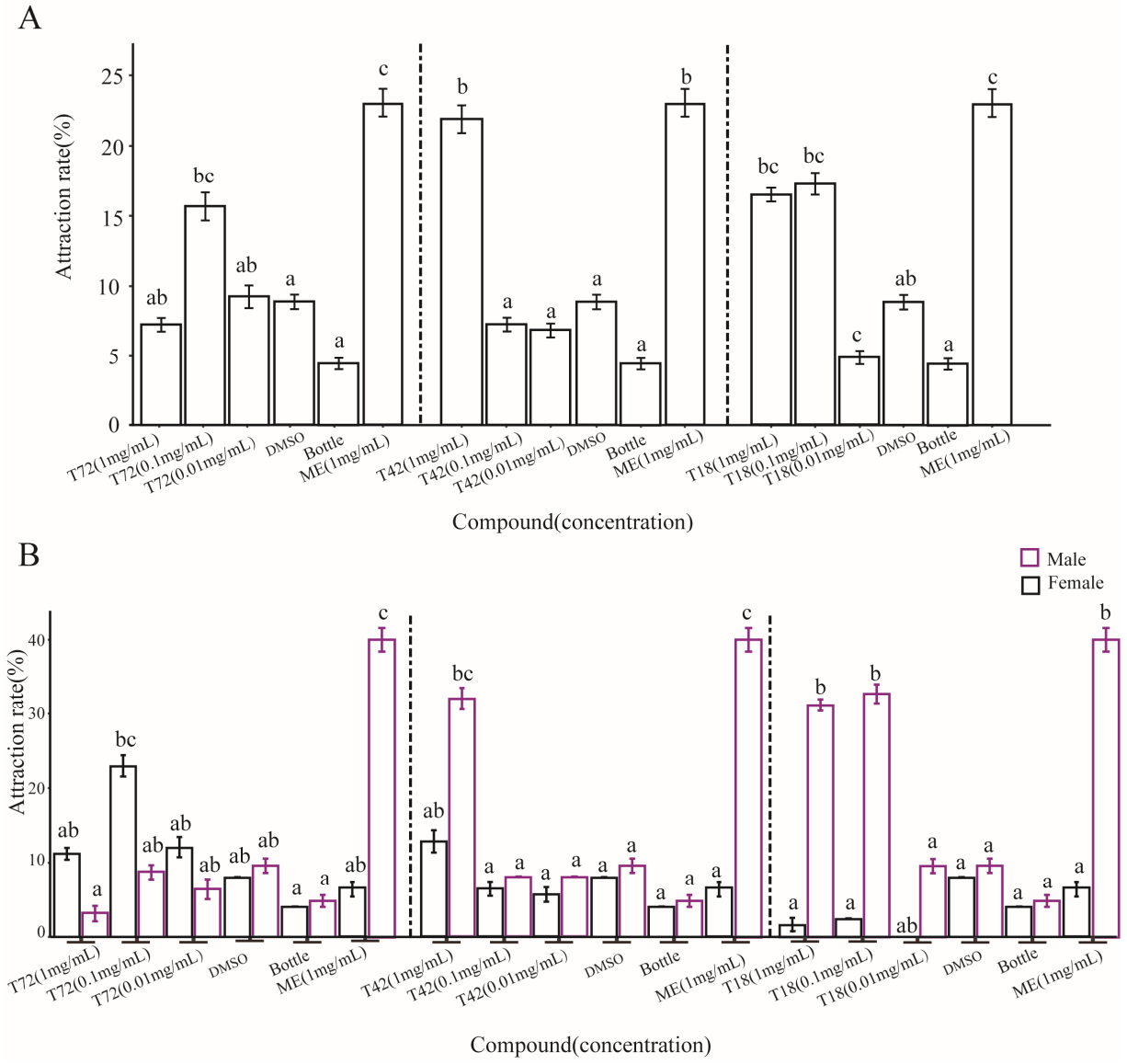

Figure 4. Olfactory preference behavior in *B. dorsalis* induced by exemestane, progesterone, 19-norandrostenedione and ME. T72 represents exemestane; T42 represents progesterone; T18 represents 19-norandrostenedione; DMSO represents the solvent; Bottle represents the empty bottle; ME represents methyl eugenol. Exemestane, progesterone, 19-norandrostenedione and ME were diluted with DMSO. The histograms show the number of olfactory preference behavior in *B. dorsalis* after 24 h and the average number of *B. dorsalis*. Data are represented by the means ± SE of five biological replicates. Statistical significance was determined using multiple comparisons ($p < 0.05$). Values with the same small letters in the bars are not significantly different at the 0.05 level. (**A**) Attraction rate of different concentrations and compounds to *B. dorsalis* adults. (**B**) Attraction rate of different concentrations and compounds to female and male *B. dorsalis*.

4. Discussions

In this study, we examined six vital sets of chemosensory receptors: IRs, ORs, CSPs, OBPs, PBPs and SNMPs. We conducted RNA sequencing and transcriptome analysis to identify 116 genes in the antennae, mouthparts, thoracic leg and female ovipositor of *B. dorsalis*. Furthermore, we extracted compounds of similar hydrocarbon compounds by integrating compounds reported to be attractive to *B. dorsalis*.

Molecular docking prediction was conducted to predict the binding relationships between the hydrocarbon compounds and the proteins of IRs, ORs, CSPs, OBPs, PBPs

and SNMPs of *B. dorsalis*, followed by network pharmacology to screen out the potential attractive compounds. Finally, ecological experiments were conducted to verify the attractant effects of the potential attractive compounds to *B. dorsalis*. The results showed that 0.10 mg/mL 19-norandrostenedione, 1.00 mg/mL progesterone and 0.10 mg/mL exemestane were significantly attractive for *B. dorsalis*. Progesterone and 19-norandrostenedione are analogues of citral. Significant disparities in the EAG reactions of mated and virgin female *B. dorsalis* to citral at a concentration of 100 μg/μL were documented [37]. These results are meaningful enough to be used for further studies of the attractant development of insects, and these substances were not reported to be attractive to insects before, making them worthy of further study in the control of *B. dorsalis*.

From the transcriptome analysis, 116 genes were identified from the antennae, mouthparts, thoracic leg and female ovipositor of *B. dorsalis*. The identification and functional analysis of its olfactory related protein gene is helpful in mastering the molecular mechanism of olfactory recognition of *B. dorsalis*. In this study, we sampled several parts where odor-related genes might have been present. We hope to find genes and important parts that may be involved in the exchange of external odors. At present, about thirty-one OBPs, five CSPs, fifty ORs, one Orco, fourteen IRs and four SNMPs have been identified from adult antennae successfully [16,38,39]. With the mapping of the *B. dorsalis* genome, more and more olfactory genes are being identified. These olfactory genes are largely involved in the recognition of chemical pheromones by *B. dorsalis* in their external environment. For example, Orco can be involved in the recognition of rhodojaponin III, an antifeedant, and citronellal, thereby inducing oviposition avoidance behavior in adult females [18]. CSP2 has also been shown to be involved in the process of rhodopsin III recognition [40].

Based on the molecular docking, it was found that the compounds T42 (progesterone) could bind the best with protein structure *c64481c04* to gene *C64481.graph_c0*, with a binding affinity of −9.42 kcal/mol, while gene *C64481.graph_c0* was identified as OR. This indicated that progesterone might have an effect on the OR of *B. dorsalis*. Previous studies have shown that OBPs (Bdor OBP2, Bdor OBP13, Bdor OBP69a and Bdor OBP83a-2), odor receptor Bdor OR88a and atypical odor receptor Bdor Orco all participate in the molecular process of ME recognition by male *B. dorsalis* [41–46]. Within the network, the degree is used to reflect the significance of the compounds or the proteins. The protein *c56889.graph_c0* has the highest degree and is identified as IR. In a previous study, female flies exhibited a decrease in IRs and ORs following mating, while males did not demonstrate such a decrease [47]. IRs and ORs potentially play a role in the recognition of sexual signals in female flies. IRs also serve a function in detecting pheromones and general odorants, being the potential useful targets in the pest management of *B. dorsalis* and other pests [16,47]. The gene *c56889.graph_c0* is estimated to be implicated in the response of *B. dorsalis* to attractants. However, further research is required to determine the exact role of this gene in *B. dorsalis*.

B. dorsalis was a notorious polyphagous pest in China, and one of the main management strategies was to use ME as a male attractant to trap *B. dorsalis* male adults [8]. Some reports have shown that the males of *B. dorsalis* feed on and are strongly attracted to ME [48]. However, some studies have also shown that there are some factors that will be involved in the effect of the attractiveness of ME to *B. dorsalis*. For example, studies involving laboratory bioassays and improving field trapping showed that feeding impacts the attraction of the sexually mature male of *B. dorsalis* to ME, while pre-fed males are less attracted to ME compared to non-pre-fed males [49]. At the same time, there are also some differences in the attraction of compound to *B. dorsalis* between the field populations and the laboratory populations. A study showed that laboratory populations are more sensitive to ME than field populations [50]. In this study, the laboratory populations, which were obtained from the field and reared in the laboratory for a long time, were used in the experiments, and the laboratory populations were more sensitive to drugs than the field populations. The field population was exposed to many compounds, so it was not sensitive to pesticides, and the sensitivity of different populations to pesticides is very different [51]. Furthermore, some traditional gene families that were reported to have an attractant effect

towards *B. dorsalis* were used in the calculations and selections of the attractive compounds to *B. dorsalis*, such as OBPs, ORs and SNMPs. We used DMSO as a solvent so as to minimize the influence of the solvent on the attractiveness of *B. dorsalis*, with the concentration of DMSO usually used and accepted in these types of experiments being less than 0.1% [34], so this concentration was also used in this study. The combinatorial use of a laboratory population and traditional gene families was more meaningful to the analysis and prediction of the attractive compounds. Furthermore, the molecular docking and calculation of the key compounds was helpful in improving the discovery of the potential attractive compounds. Obviously, there are many factors that will have an effect on the attractants of *B. dorsalis*, such as differences in geographical populations [52], some genes besides OBPs and experiments with field populations in the field. It is meaningful to include more factors in the calculation, screening and identification of potential attractive compounds in order to find better attractants using comprehensive and accurate methods in further studies in the future.

B. dorsalis is a major agricultural pest that causes significant economic damage to fruit and vegetable crops in tropical and subtropical regions. The management strategies of *B. dorsalis* consist mainly of targeting the adult population because the larvae have cryptic feeding habits and pupate in the soil and are therefore hard to be controlled [5]. However, conventional practices for creating luring agents are costly and time-consuming in the management of adults. In this study, a compressive method that comprises combining transcriptomics, molecular docking, network pharmacology and behavioral assays is proposed. It is meaningful in the discovery of the attractants of *B. dorsalis* in an efficiency and accurate way.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10030299/s1, Figure S1: Identification of Olfactory Sensory Proteins in *B. dorsalis*. (A) Phylogenetic trees of CSP genes. (B) Phylogenetic trees of OBPGOBP genes. (C) Phylogenetic trees of OR genes. (D) Phylogenetic trees of PBP genes. (E) Phylogenetic trees of SNMP genes. (F) Phylogenetic trees of IR genes. The identified genes of *B. dorsalis* are highlighted in blue. The abbreviated names of species: Apisu: *Acyrthosiphon pisum*; Bcuc: *Bactrocera cucurbitae*; Zcuc: *Zeugodacus cucurbitae*; Bdor: *Bactrocera dorsalis*; Bmori: *Bombyx mori*; Bole: *Bactrocera oleae*; Ccap: *Ceratitis capitata*; Dana: *Drosophila ananassae*; Dere: *Drosophila erecta*; Dgri: *Drosophila grimshawi*; Dmel: *Drosophila melanogaster*; Dmoj: *Drosophila mojavensis*; Dper: *Drosophila persimilis*; Dpse: *Drosophila pseudoananassae*; Dsec: *Drosophila sechellia*; Dsim: *Drosophila simulans*; Dvir: *Drosophila virilis*; Dwil: *Drosophila willistoni*; Dyak: *Drosophila Yakuba*; Table S1: Olfactory Receptor Genes Identified.txt; Table S2: Summary Classification of Potential Attractive Compounds.xlsx; Table S3: Similarity Compounds Corresponding to Hydrocarbon Potential Attracitiveness.xlsx; Table S4: Docking Results.txt; Table S5: The Degree Table Corresponding to the GPGP Network.csv.

Author Contributions: Conceptualization, Y.L. (Yongyue Lu), G.L. and D.W.; data curation, Y.C., F.C. and Y.Z.; investigation, Y.Z., Y.C., D.W., Y.F., H.S., X.B., X.X., L.Z., Y.L. (Yongyue Lu) and F.C.; methodology, Y.L. (Yongyue Lu), G.L., Y.C. and F.C.; project administration, Y.L. (Yongyue Lu) and F.C.; validation, Y.Z. and Y.C.; visualization, Y.C., Y.Z., Y.L. (Yi Li) and J.W.; writing—original draft, Y.Z., Y.C. and F.C.; writing—review and editing, Y.C., Y.L. (Yongyue Lu), F.C., Y.Z., D.W. and G.L. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in the study are included in the article and Supplementary Material.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

Apisu	*Acyrthosiphon pisum*
B. dorsalis	*Bactrocera dorsalis*
Bcuc	*Bactrocera cucurbitae*
Bcuc	*Zeugodacus cucurbitae*
Bdor	*Bactrocera dorsalis*
Bmori	*Bombyx mori*
Bole	*Bactrocera oleae*
Ccap	*Ceratitis capitata*
CPGP network	Compound–pathway–GO term–olfactory sensory protein network
CSP	Chemosensory protein
Dana	*Drosophila ananassae*
Dere	*Drosophila erecta*
Dgri	*Drosophila grimshawi*
Dmel	*Drosophila melanogaster*
Dmoj	*Drosophila mojavensis*
Dper	*Drosophila persimilis*
Dpse	*Drosophila pseudoananassae*
Dsec	*Drosophila sechellia*
Dsim	*Drosophila simulans*
Dvir	*Drosophila virilis*
Dwil	*Drosophila willistoni*
Dyak	*Drosophila Yakuba*
GO	Gene ontology
GR	Gustatory receptor
IR	Ionotropic receptor
KEGG	Kyoto Encyclopedia of Genes and Genomes
ME	Methyl eugenol
MP	Maximum parsimony
OBP	Odorant-binding protein
ORF	Open reading frame
OR	Olfactory receptor
PBP	Pheromone-binding protein
SPR	Subtree pruning and regrafting
SNMP	Sensory neuron membrane protein
TCM	Traditional Chinese Medicine

References

1. Schutze, M.K.; Bourtzis, K.; Cameron, S.L.; Clarke, A.R.; De Meyer, M.; Hee, A.K.W.; Hendrichs, J.; Krosch, M.N.; Mwatawala, M. Integrative Taxonomy versus Taxonomic Authority without Peer Review: The Case of the Oriental Fruit Fly, *Bactrocera dorsalis* (Tephritidae). *Syst. Entomol.* **2017**, *42*, 609–620. [CrossRef]
2. Vargas, R.I.; Pinero, J.C.; Leblanc, L. An Overview of Pest Species of Bactrocera Fruit Flies (Diptera: Tephritidae) and the Integration of Biopesticides with Other Biological Approaches for Their Management with a Focus on the Pacific Region. *Insects* **2015**, *6*, 297–318. [CrossRef] [PubMed]
3. Jiang, F.; Liang, L.; Wang, J.; Zhu, S. Chromosome-Level Genome Assembly of *Bactrocera dorsalis* Reveals Its Adaptation and Invasion Mechanisms. *Commun. Biol.* **2022**, *5*, 25.
4. Yu, C.; Zhao, R.; Zhou, W.; Pan, Y.; Tian, H.; Yin, Z.; Chen, W. Fruit Fly in a Challenging Environment: Impact of Short-Term Temperature Stress on the Survival, Development, Reproduction, and Trehalose Metabolism of *Bactrocera dorsalis* (Diptera: Tephritidae). *Insects* **2022**, *13*, 8. [CrossRef] [PubMed]
5. Dong, Z.; He, Y.; Ren, Y.; Wang, G.; Chu, D. Seasonal and Year-Round Distributions of *Bactrocera dorsalis* (Hendel) and Its Risk to Temperate Fruits under Climate Change. *Insects* **2022**, *13*, 550. [CrossRef]
6. Dias, N.P.; Zotti, M.J.; Montoya, P.; Carvalho, I.R.; Nava, D.E. Fruit Fly Management Research: A Systematic Review of Monitoring and Control Tactics in the World. *Crop Prot.* **2018**, *112*, 187–200. [CrossRef]
7. Tan, K.H.; Nishida, R. Methyl Eugenol: Its Occurrence, Distribution, and Role in Nature, Especially in Relation to Insect Behavior and Pollination. *J. Insect Sci.* **2012**, *12*, 56. [CrossRef]
8. Fan, Y.; Zhang, C.; Qin, Y.; Yin, X.; Dong, X.; Desneux, N.; Zhou, H. Monitoring the Methyl Eugenol Response and Non-Responsiveness Mechanisms in Oriental Fruit Fly *Bactrocera dorsalis* in China. *Insects* **2022**, *13*, 1004. [CrossRef]

9. Wang, Y.; Gao, X.; Zhang, B.; Cheng, Y. Building Methodology for Discovering and Developing Chinese Medicine Based on Network Biology. *Zhongguo Zhong Yao Za Zhi = Zhongguo Zhongyao Zazhi = China J. Chin. Mater. Medica* **2011**, *36*, 228–231.
10. Wu, L.; Wang, Y.; Fan, X. Tools for Network Pharmacology Study: Network Visualization and Network Analysis. *Zhongguo Zhong Yao Za Zhi = Zhongguo Zhongyao Zazhi = China J. Chin. Mater. Medica* **2011**, *36*, 2923–2925.
11. Wang, Z.-Y.; Wang, X.; Zhang, D.-Y.; Hu, Y.-J.; Li, S. Traditional Chinese Medicine Network Pharmacology: Development in New Era under Guidance of Network Pharmacology Evaluation Method Guidance. *Zhongguo Zhong Yao Za Zhi = Zhongguo Zhongyao Zazhi = China J. Chin. Mater. Medica* **2022**, *47*, 7–17. [CrossRef]
12. Sun, J.S.; Xiao, S.; Carlson, J.R. The Diverse Small Proteins Called Odorant-Binding Proteins. *R. Soc. Open Biol.* **2018**, *8*, 180208. [CrossRef]
13. Zhao, Y.J.; Li, G.C.; Zhu, J.Y.; Liu, N.Y. Genome-Based Analysis Reveals a Novel SNMP Group of the Coleoptera and Chemosensory Receptors in Rhaphuma Horsfieldi. *Genomics* **2020**, *112*, 2713–2728. [CrossRef] [PubMed]
14. Robertson, H.M. Molecular Evolution of the Major Arthropod Chemoreceptor Gene Families. *Annu. Rev. Entomol.* **2019**, *64*, 227–242. [CrossRef] [PubMed]
15. Karpe, S.D.; Tiwari, V.; Ramanathan, S. InsectOR-Webserver for Sensitive Identification of Insect Olfactory Receptor Genes from Non-Model Genomes. *PLoS ONE* **2021**, *16*, e0245324. [CrossRef] [PubMed]
16. Wu, Z.; Zhang, H.; Wang, Z.; Bin, S.; He, H.; Lin, J. Discovery of Chemosensory Genes in the Oriental Fruit Fly, *Bactrocera dorsalis*. *PLoS ONE* **2015**, *10*, e0129794. [CrossRef] [PubMed]
17. Yao, R.; Zhao, M.; Zhong, L.; Li, Y.; Li, D.; Deng, Z.; Ma, X. Characterization of the Binding Ability of the Odorant Binding Protein BminOBP9 of Bactrocera Minax to Citrus Volatiles. *Pest Manag. Sci.* **2021**, *77*, 1214–1225. [CrossRef]
18. Yi, X.; Zhao, H.; Wang, P.; Hu, M.; Zhong, G. Bdor\Orco is Important for Oviposition-Deterring Behavior Induced by Both the Volatile and Non-Volatile Repellents in *Bactrocera dorsalis* (Diptera: Tephritidae). *J. Insect Physiol.* **2014**, *65*, 51–56. [CrossRef] [PubMed]
19. Cai, P.; Hong, J.; Wang, C.; Yang, Y.; Zhang, Q.; Ji, Q.; Chen, J. Radiation of *Bactrocera dorsalis* (Diptera: Tephritidae) Eggs to Improve the Mass Rearing of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). *J. Econ. Entomol.* **2018**, *111*, 1157–1164. [CrossRef] [PubMed]
20. Altschul, S.F.; Madden, T.L.; Schäffer, A.A.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D.J. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs. *Nucleic Acids Res.* **1997**, *25*, 3389–3402. [CrossRef]
21. Götz, S.; García-Gómez, J.M.; Terol, J.; Williams, T.D.; Nagaraj, S.H.; Nueda, M.J.; Robles, M.; Talón, M.; Dopazo, J.; Conesa, A. High-Throughput Functional Annotation and Data Mining with the Blast2go Suite. *Nucleic Acids Res.* **2008**, *36*, 3420–3435. [CrossRef] [PubMed]
22. Conesa, A.; Götz, S.; García-Gómez, J.M.; Terol, J.; Talón, M.; Robles, M. Blast2go: A Universal Tool for Annotation, Visualization and Analysis in Functional Genomics Research. *Bioinformatics* **2005**, *21*, 3674–3676. [CrossRef] [PubMed]
23. Xu, Y.L.; He, P.; Zhang, L.; Fang, S.Q.; Dong, S.L.; Zhang, Y.J.; Li, F. Large-Scale Identification of Odorant-Binding Proteins and Chemosensory Proteins from Expressed Sequence Tags in Insects. *BMC Genom.* **2009**, *10*, 632. [CrossRef] [PubMed]
24. Petersen, T.N.; Brunak, S.; von Heijne, G.; Nielsen, H. SignalP 4.0: Discriminating Signal Peptides from Transmembrane Regions. *Nat. Methods* **2011**, *8*, 785–786. [CrossRef] [PubMed]
25. Krogh, A.; Larsson, B.; von Heijne, G.; Sonnhammer, E.L. Predicting Transmembrane Protein Topology with a Hidden Markov Model: Application to Complete Genomes. *J. Mol. Biol.* **2001**, *305*, 567–580. [CrossRef] [PubMed]
26. Katoh, K.; Toh, H. Parallelization of the MAFFT Multiple Sequence Alignment Program. *Bioinformatics* **2010**, *26*, 1899–1900. [CrossRef] [PubMed]
27. Vieira, F.G.; Rozas, J. Comparative Genomics of the Odorant-Binding and Chemosensory Protein Gene Families across the Arthropoda: Origin and Evolutionary History of the Chemosensory System. *Genome Biol. Evol.* **2011**, *3*, 476–490. [CrossRef]
28. Siciliano, P.; Scolari, F.; Gomulski, L.M.; Falchetto, M.; Manni, M.; Gabrieli, P.; Field, L.M.; Zhou, J.J.; Gasperi, G.; Malacrida, A.R. Sniffing Out Chemosensory Genes from the Mediterranean Fruit Fly, *Ceratitis capitata*. *PLoS ONE* **2014**, *9*, e85523. [CrossRef]
29. Yu, G.; Wang, L.G.; Han, Y.; He, Q.Y. Clusterprofiler: An R Package for Comparing Biological Themes among Gene Clusters. *Omics* **2012**, *16*, 284–287. [CrossRef]
30. Kanehisa, M.; Sato, Y. KEGG Mapper for Inferring Cellular Functions from Protein Sequences. *Protein Sci.* **2020**, *29*, 28–35. [CrossRef]
31. Li, J.; Fu, A.; Zhang, L. An Overview of Scoring Functions Used for Protein-Ligand Interactions in Molecular Docking. *Interdiscip. Sci.* **2019**, *11*, 320–328. [CrossRef]
32. Elhenawy, A.A.; Al-Harbi, L.M.; El-Gazzar, M.A.; Khowdiary, M.M.; Ouidate, A.; Alosaimi, A.M.; Elhamid Salim, A. Naproxenylamino Acid Derivatives: Design, Synthesis, Docking, QSAR and Anti-Inflammatory and Analgesic Activity. *Biomed. Pharmacother.* **2019**, *116*, 109024. [CrossRef]
33. Wu, B.; Lan, X.; Chen, X.; Wu, Q.; Yang, Y.; Wang, Y. Researching the Molecular Mechanisms of Taohong Siwu Decoction in the Treatment of Varicocele-Associated Male Infertility Using Network Pharmacology and Molecular Docking: A Review. *Medicine* **2023**, *102*, e34476. [CrossRef] [PubMed]
34. Wu, S.X.; Chen, Y.; Lei, Q.; Peng, Y.Y.; Jiang, H.B. Sublethal Dose of β-Cypermethrin Impairs the Olfaction of *Bactrocera dorsalis* by Suppressing the Expression of Chemosensory Genes. *Insects* **2022**, *13*, 721. [CrossRef] [PubMed]

35. George, L.F.; Pradhan, S.J.; Mitchell, D.; Josey, M.; Casey, J.; Belus, M.T.; Fedder, K.N.; Dahal, G.R.; Bates, E.A. Ion Channel Contributions to Wing Development in Drosophila melanogaster. *G3 Genes Genomes Genet.* **2019**, *9*, 999–1008. [CrossRef]
36. Prelic, S.; Getahun, M.N.; Kaltofen, S.; Hansson, B.S.; Wicher, D. Modulation of the No-cGMP Pathway Has No Effect on Olfactory Responses in the *Drosophila* antenna. *Front. Cell. Neurosci.* **2023**, *17*, 1180798. [CrossRef] [PubMed]
37. Zhang, G.; Wang, J. Electrophysiological responses of the oriental fruit fly, *Bactrocera dorsalis* to host-plant related volatiles. *J. Environ. Entomol.* **2016**, *38*, 126–131.
38. Zheng, W.; Peng, W.; Zhu, C.; Zhang, Q.; Saccone, G.; Zhang, H. Identification and Expression Profile Analysis of Odorant Binding Proteins in the Oriental Fruit Fly *Bactrocera dorsalis*. *Int. J. Mol. Sci.* **2013**, *14*, 14936–14949. [CrossRef] [PubMed]
39. Liu, Z.; Smagghe, G.; Lei, Z.; Wang, J.J. Identification of Male- and Female-Specific Olfaction Genes in Antennae of the Oriental Fruit Fly (*Bactrocera dorsalis*). *PLoS ONE* **2016**, *11*, e0147783. [CrossRef]
40. Yi, X.; Wang, P.; Wang, Z.; Cai, J.; Hu, M.; Zhong, G. Involvement of a Specific Chemosensory Protein from *Bactrocera dorsalis* in Perceiving Host Plant Volatiles. *J. Chem. Ecol.* **2014**, *40*, 267–275. [CrossRef]
41. Liu, H.; Zhao, X.F.; Fu, L.; Han, Y.Y.; Chen, J.; Lu, Y.Y. BdorOBP2 Plays an Indispensable Role in the Perception of Methyl eugenol by Mature Males of *Bactrocera dorsalis* (Hendel). *Sci. Rep.* **2017**, *7*, 15894. [CrossRef]
42. Liu, H.; Chen, Z.S.; Zhang, D.J.; Lu, Y.Y. BdorOR88a Modulates the Responsiveness to Methyl Eugenol in Mature Males of *Bactrocera dorsalis* (Hendel). *Front. Physiol.* **2018**, *9*, 987. [CrossRef] [PubMed]
43. Liu, H.; Wang, D.D.; Wan, L.; Hu, Z.Y.; He, T.T.; Wang, J.B.; Deng, S.Z.; Wang, X.S. Assessment of Attractancy and Safeness of (E)-Coniferyl Alcohol for Management of Female Adults of Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel). *Pest Manag. Sci.* **2022**, *78*, 1018–1028. [CrossRef] [PubMed]
44. Chen, X.; Lei, Y.; Li, H.; Xu, L.; Yang, H.; Wang, J.; Jiang, H. CRISPR/Cas9 Mutagenesis Abolishes Odorant-Binding Protein BdorOBP56f-2 and Impairs the Perception of Methyl Eugenol in *Bactrocera dorsalis* (Hendel). *Insect Biochem. Mol. Biol.* **2021**, *139*, 103656. [CrossRef] [PubMed]
45. Chen, X.; Yang, H.; Wu, S.; Zhao, W.; Hao, G.; Wang, J.; Jiang, H. BdorOBP69a Is Involved in the Perception of the Phenylpropanoid Compound Methyl Eugenol in Oriental Fruit Fly (*Bactrocera dorsalis*) Males. *Insect Biochem. Mol. Biol.* **2022**, *147*, 103801. [CrossRef] [PubMed]
46. Xu, L.; Jiang, H.B.; Tang, K.Y.; Yan, Y.; Schetelig, M.F.; Wang, J.J. CRISPR-Mediated Mutagenesis of the Odorant Receptor co-Receptor (Orco) Gene Disrupts Olfaction-Mediated Behaviors in *Bactrocera dorsalis*. *Insect Sci.* **2022**, *29*, 1275–1286. [CrossRef] [PubMed]
47. Jin, S.; Zhou, X.; Gu, F.; Zhong, G.; Yi, X. Olfactory Plasticity: Variation in the Expression of Chemosensory Receptors in *Bactrocera dorsalis* in Different Physiological States. *Front. Physiol.* **2017**, *8*, 672. [CrossRef] [PubMed]
48. Kah-Wei Hee, A.; Tan, K.H. Transport of Methyl Eugenol-Derived Sex Pheromonal Components in the Male Fruit Fly, *Bactrocera dorsalis*. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2006**, *143*, 422–428. [CrossRef] [PubMed]
49. Rasool, A.; Fatima, S.; Shah, S.H.; Munis, M.F.H.; Irshad, A.; Shelly, T.E.; Haq, I.U. Methyl Eugenol Aromatherapy: A Delivery System Facilitating the Simultaneous Application of Male Annihilation and Sterile Insect Technique against the Peach Fruit Fly. *Pest Manag. Sci.* **2024**, *80*, 1465–1473. [CrossRef]
50. Sim, S.B.; Curbelo, K.M.; Manoukis, N.C.; Cha, D.H. Evaluating *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) Response to Methyl Eugenol: Comparison of Three Common Bioassay Methods. *J. Econ. Entomol.* **2022**, *115*, 556–564. [CrossRef]
51. Buczkowski, G. A comparison of Insecticide Susceptibility Levels in 12 Species of urban Pest Ants with Special Focus on the Odorous House Ant, Tapinoma Sessile. *Pest Manag. Sci.* **2021**, *77*, 2948–2954. [CrossRef] [PubMed]
52. Nugnes, F.; Russo, E.; Viggiani, G.; Bernardo, U. First Record of an Invasive Fruit Fly Belonging to *Bactrocera dorsalis* Complex (Diptera: Tephritidae) in Europe. *Insects* **2018**, *9*, 182. [CrossRef] [PubMed]