

Review

A Review on Digestive System of *Rhynchophorus ferrugineus* as Potential Target to Develop Control Strategies

Ahmad-Faris Seman-Kamarulzaman ^{1,2}, Faizatul Atikah Pariamiskal ¹, Amiratul Nabihah Azidi ¹ and Maizom Hassan ^{1,*}

¹ Institute of Systems Biology, Universiti Kebangsaan Malaysia, Bangi 43600, Selangor, Malaysia; afkamarulzaman@uitm.edu.my (A.-F.S.-K.); p115980@siswa.ukm.edu.my (F.A.P.)

² Faculty of Applied Sciences, Universiti Teknologi MARA Pahang, Bandar Tun Abdul Razak Jengka 26400, Pahang, Malaysia

* Correspondence: maizom@ukm.edu.my

Simple Summary: The red palm weevil poses a significant threat to palm species, resulting in substantial economic losses. While multiple methods have been developed to control its infestations, there is an urgent need for eco-friendly insecticides that selectively target its critical systems or pathways. One such target is its digestive system, which is essential for its survival. This review highlights the potential of using the digestive system of the red palm weevil to manage its infestations. Proteomic and transcriptomic data analyses on the weevils have provided a better understanding of the protein and gene compositions in its digestive system. With technological advancements, a more comprehensive approach can be taken to explore the opportunities in manipulating the data on the digestive system of red palm weevil, leading to improved management methods.

Abstract: *Rhynchophorus ferrugineus*, commonly known as red palm weevil (RPW), is a high-risk insect pest that has become a threat to many important palm species. There are several dominant factors that lead to the successful infestation of RPW, including its stealthy lifestyle, highly chitinized mouthpart, and high fecundity rate. Due to that, millions of dollars of losses have been suffered by many countries invaded by RPW. Several methods have been designed to control its invasion, including the usage of insecticides, but many cause resistance and environmental pollution. Therefore, an environmentally friendly insecticide that targets specific systems or pathways in RPW is urgently needed. One of the potential targets is the digestive system of RPW, as it is the major interface between the insect and its plant host. The related knowledge of RPW's digestive system, such as the anatomy, microflora, transcriptomic analysis, and proteomic analysis, is important to understand its effects on RPW's survival. Several data from different omics regarding the digestive systems of RPW have been published in separate reports. Some of the potential targets have been reported to be inhibited by certain potential insecticides, while other targets have not yet been tested with any inhibitors. Hence, this review may lead to a better understanding on managing infestations of RPW using the system biology approach for its digestive system.

Keywords: red palm weevil; gut; potential approach; pest management; omics



Citation: Seman-Kamarulzaman, A.-F.; Pariamiskal, F.A.; Azidi, A.N.; Hassan, M. A Review on Digestive System of *Rhynchophorus ferrugineus* as Potential Target to Develop Control Strategies. *Insects* **2023**, *14*, 506. <https://doi.org/10.3390/insects14060506>

Academic Editors: Camilo Ayra-Pardo and Denis J. Wright

Received: 26 April 2023

Revised: 21 May 2023

Accepted: 27 May 2023

Published: 31 May 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Rhynchophorus ferrugineus (Coleoptera: Curculionidae), commonly known as the red palm weevil (RPW) (Olivier 1790), is a Coleopteran insect that has been classified as a significantly serious pest on the A2 list (pests are locally present), according to the European and Mediterranean Plant Protection Organization (EPPO) [1]. RPW has been reported to invade various economically important palm species on almost all continents, except Antarctica [2,3]. Their invasion has cost 30% of the world's date palm (*Phoenix dactylifera*) production loss in the Middle East region, with an estimation of 130 million

USD annually [4]. In addition, the infestation of this pest has caused fatal damage to nearly 200,000 young coconut palms (*Cocos nucifera* L.) in Sri Lanka, resulting in a financial loss of about 1.8 million USD in 2005 [5]. In Malaysia, RPW infestations have rapidly spread in coconut plantation areas in Terengganu, from 58 localities in 2007 [6] to 858 localities in 2011. Their infestation was further reported to have spread to three other states in the northern region of Peninsular Malaysia in 2016.

RPW is a sexually dimorphic [7] and holometabolous insect, as it has four developmental stages that consist of egg, larvae, pupae and adult (Figure 1) [8]. It takes about 45 to 298 days to complete the whole lifecycle, depending on their diet [9]. The female RPW has a high fecundity rate, producing around 180–396 eggs throughout its lifespan [10].

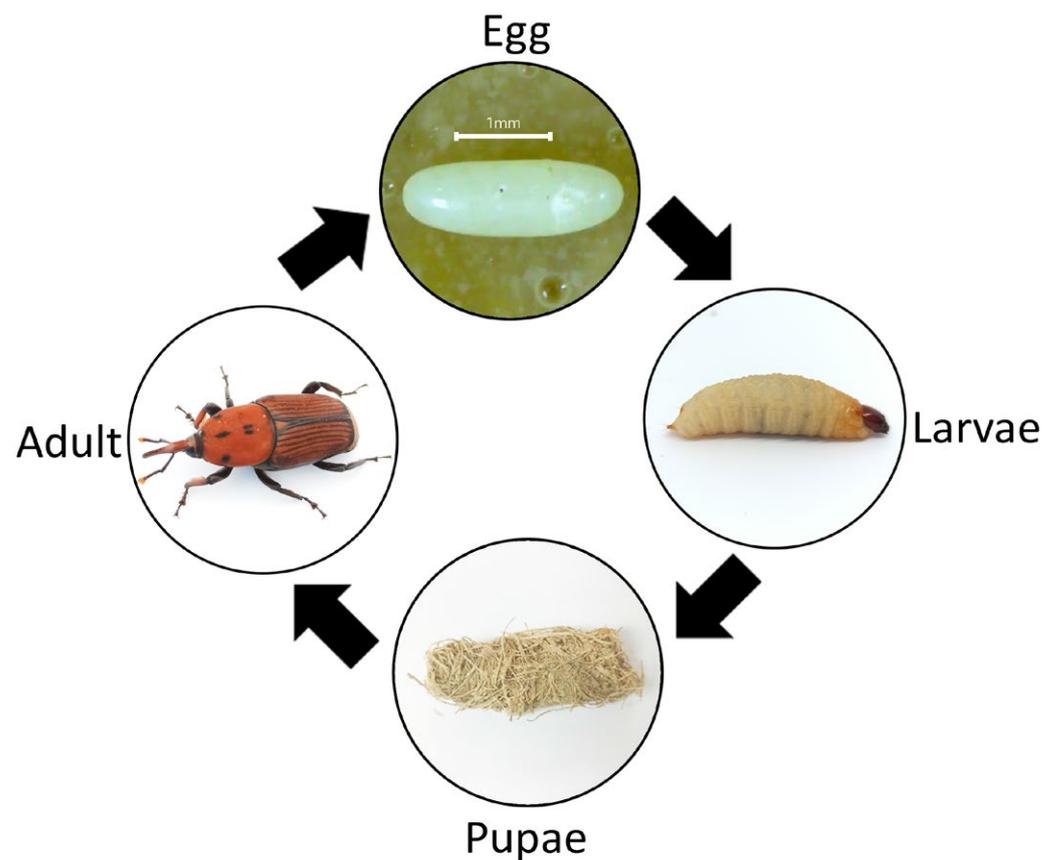


Figure 1. Lifecycle of RPW as holometabolous insects.

RPW has been reported to be most destructive in its larval stage [11]. The larvae feed on the trunk, creating empty cavities inside and leading to the death of the tree. It is difficult to identify infested palm trees in palm plantations due to the lack of obvious symptoms of infestation, especially at the early stage of infestation [3,12]. The remarkable adaptation of the concealed RPW's lifecycle also makes it hard to remove the pest from the palm tree besides dissection [7]. The damage from the RPW infestation is fatal, as the physical symptoms are only visible after the tree has been severely damaged, thus destroying the tree beyond saving before the pest is detected [1].

The current strategies reported for the prevention and control of RPW are based on a natural enemy as a biocontrol agent, food-baited pheromone traps and insecticides [6]. Out of the various choices, broad-spectrum insecticides are currently the main strategy for RPW control [13–16]. However, the usage of commercial broad-spectrum insecticides, such as phosphine [17] and ethion [18], has been proven ineffective, as RPW develops a resistance towards them [16]. Moreover, they pollute the environment, and they kill nontargeted beneficial insects [4]. Accordingly, the insufficient current management [19] leads to the

need for in-depth studies for RPW control, such as using molecular approaches that target its specific biochemical system. One of the potential systems is the digestive system of RPW, as it is one of the ways to control its infestation, but little is known about it [13,20].

Hence, this article aimed to review the available information regarding RPW's digestive system. The knowledge related to RPW's digestive system, such as its anatomy, microflora, transcriptomic analysis, and proteomic analysis, especially factors that may affect its feeding behavior, could be the first step in exploring the possibility of new treatments to manage RPW infestations in palm tree species [12].

2. Anatomy of RPW's Digestive System

Within the body system of an insect, the most crucial one in the insect's life is the digestive tract system. The digestive tract is basically responsible for supplying the essential nutrients for survival and growth, as well as carrying out daily activities [15]. RPW feeds on the fibrous part of the host plant using its mouthpart. One of the causes that leads to a successful infestation of RPW is its highly chitinous mouthpart [21]. As a phytophagous insect that feeds on palm species, RPW mouthparts consist of strong mandibles with a bite-chew mechanism. The characteristics of the mouthpart enable RPW to drill into the stem of the host plant. This is different from the mouthparts of beneficial insects, such as pollinators (Hymenoptera) that utilize fluid-feeding mechanism using a proboscis [22].

When the damage due to infestation worsens, a chewing sound can be heard when one's ear is close to the palm trunk [23]. In addition to the RPW's strong mouthpart, this economically important pest also possesses an alimentary gut system that can digest the high-fiber food that it ingests. The gut can be divided into three different parts (Figure 2): foregut, midgut and hindgut [13,15,24]. Mainly, the foregut is used to eat the food, and the hindgut is for the absorption of water. The secretion of enzymes and digestion of food occur in the midgut [13].

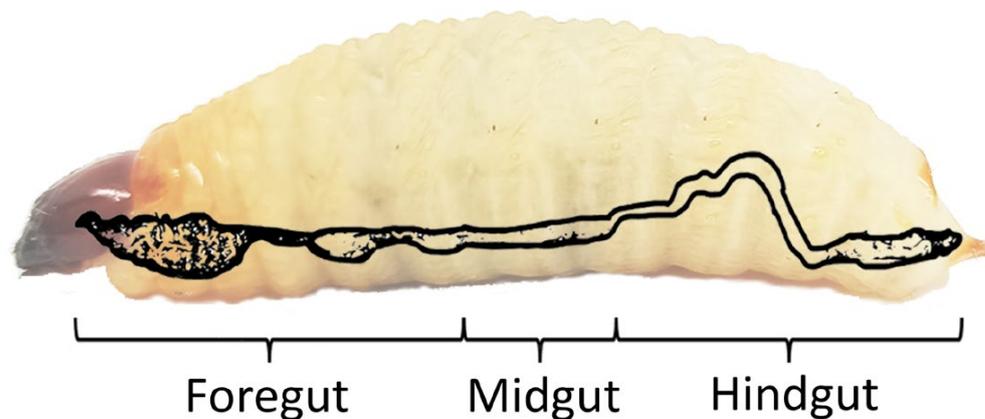


Figure 2. Internal digestive organ of an adult red palm weevil, *Rhynchophorus ferrugineus*. The gut is divided into three parts: foregut, midgut and hindgut.

The digestive organ of RPW is reported as slightly different between larvae and adults due to the differences in their feeding modes [13]. According to Harris et al. [15], the larvae have larger organs, as they consume more food and need more energy for periodic molting process. Any hindrance to the digestive system of the larvae may result in the death of the larvae due to nutrient deficiency and insufficient energy. Hence, the gut in larvae can become a great target to control RPW infestations.

3. Gut Bacteria of RPW

Other than the anatomy of the gut, the content of the gut is also similarly important, especially the microflora which greatly helps to enhance food digestion in RPW. It was reported that insect gut microbiota was generally dominated by *Proteobacteria* and *Bacteroidetes* [25–27]. One study revealed that the gut bacteria present in RPW exist in six

abundant groups of bacteria with different phylogenetic group ranks: *Enterobacteriaceae*, *Leminorella grimontii*, *Entomoplasmatales*, *Erysipelothrix*, *Lactobacillus* and *Leuconostoc* [28]. However, this composition and population dynamics can vary due to environmental factors, including the diet of the insect [29].

The gut microbiota was reported to promote effects on the growth, development, mating and immunity of RPW larvae [30,31]. RPW's gut was also reported to be equipped with bacteria that provided tyrosine, which is essential for the formation of the insect's cuticle and hardening [32]. An infection of aposymbiotic RPW with *Serratia marcescens* and *Escherichia coli* showed a significantly faster rate of death compared to the symbiotic RPW. Therefore, the gut microbiota was proven to benefit RPW in its survival and immune defense [31]. In addition, the gut bacteria were reported to be able to manipulate the way in which RPW processed and utilized nutrients. It was shown that the body mass, protein content and glucose levels were higher in conventionally reared RPW compared to germ-free RPW [33].

To further investigate the microbiota present in the gut of beetles, especially RPW, various approaches involving metagenomic studies can be utilized [34]. Some of the approaches are 16S rRNA gene sequencing [35], shotgun metagenomics [36] and meta-transcriptomics [37]. The usage of the 16S rRNA gene sequencing approach was reported to successfully identify seven potential gut bacteria to be targeted for the management of RPW. The gut bacteria identified were *Serratia enterica*, *Enterobacter cloacae*, *Raoultella* sp., *Klebsiella pneumoniae*, *Klebsiella variicola*, *Klebsiella oxytoca* and *Citrobacter koseri*. Those seven bacteria were determined to have a great cellulose degradation ability. Disrupting the symbiosis between RPW and the bacteria may result in a significantly affected nutrition metabolism of the RPW [35].

The utilization of the shotgun metagenomics approach on RPW was reported to identify the two most abundant species, namely *Klebsiella pneumoniae* and *Lactococcus lactis* [36]. *K. pneumoniae* was also found in the spruce bark beetle *Dendroctonus micans* [38], and it has been suggested to be involved in cellulolytic digestion [39]. *L. lactis*, a type of lactic acid bacteria, plays a significant role in the digestion and fermentation of plant polymers in the gut of RPW to enhance the insect's ability to obtain nutrition from its diet. Moreover, *L. lactis* carries out lactic acid fermentation, which converts carbohydrates into lactic acid or lactate. This helps to break down carbohydrates into a more usable form for the insect and maintains an acidic environment in the gut, promoting the growth of beneficial bacteria and inhibiting the growth of harmful bacteria [33]. Both bacteria were suggested to be potential candidates for further studies on gut indigestion in RPW. Other than that, a pathogenic virus called *Cotesia sesamiae* bracovirus (CsBV) was also discovered in RPW's gut in the same study [36]. CsBV is a type of virus that belongs to the *Polydnaviridae* family. It is a symbiotic virus that is carried by the parasitic wasp *C. sesamiae* and is injected into the host insect when the wasp lays its eggs inside the host's body [40]. Previously, the cytoplasmic polyhedrosis virus has been reported to affect RPW, resulting in malformed adults [41]. Hence, Jia et al. [36] suggested that the insect-infectious bracovirus is worth being further studied as a candidate pathogen for effective RPW biocontrol. In addition, host-borne bacteriophages were identified in the gut of RPW [36]. Several bacteriophages have been known to cause mortality in RPW larvae [42,43], and therefore, the identified bacteriophage could also infect a range of gut bacterial species and cause an imbalance in RPW's gut microbiota population [36].

Meanwhile, the metatranscriptomic approach, which can identify actively expressed genes in complex microbial communities, has been used to study the gut microbiota of beetles but not yet in RPW [37]. The metatranscriptomic approach was used, as it is a powerful tool that can reveal the actively expressed genes in complex microbial communities [44]. The flea beetles, namely *Altica fragariae* and *A. viridicyanea*, feeding on *Duchesnea indica* and *Geranium nepalense*, respectively, were compared after being swapped between hosts, revealing differences in their microbial communities and enriched genes identified in the gut [37]. These genes were involved in breaking down the secondary metabolites produced

by the host plant, and the microbial communities were found to support their function. The gut microbiota helped the beetle to feed on and adapt to their potentially toxic host plants, highlighting the link between the functions of these genes and the diversity of the microbial communities [37]. The link between the diversity of gut microbiota and gene functions was also shown in grasshoppers [45]. Therefore, applying the metatranscriptomic approach to RPW's gut could reveal the potential gut microbiota that cooperates with RPW, contributing to understanding its adaptation to different host plants and potentially managing its survival [37]. The cooperation between the insect's gut microbiota, which leads to a better metabolism for the host's nutrition, has also been shown in sugarcane borer *Diatraea saccharalis* [46]. Zhang et al. suggested that the understanding in the host adaptation could be explored by the utilization of an artificial feeding system, incorporating in vitro cultured gut wall cells to replicate the insect gut environment. This system allows for the assessment of host nutrition–gut microbiota interactions [47]. In summary, exploring the dynamics of the microbiota diversity present in the gut of RPW using various approaches in the metagenomic analysis is crucial for developing effective biocontrol strategies to manage this pest [11]. It is a promising alternative method to control this pest by disrupting the interactions between RPW and its gut microbiota [33,35].

4. Protein Profile of RPW's Gut

Another important component in the digestive system of RPW besides the microflora is the digestive enzyme. Digestive enzymes play an essential role for proper and efficient digestive system function (Table 1). The presence of certain digestive enzymes in the system enables RPW to digest the foods it consumes. A thorough study about digestive enzymes in their gut enabled the discovery of the relationship between the insect and its environment, indicating the degree of food preference. Therefore, identifying digestive enzymes through liquid chromatography-mass spectrometry (LC-MS) from the protein profile of the RPW gut reared on different diets, namely coconuts, oil palm and sago, can provide insights into the preference of the host plant for an RPW infestation. This approach is valuable for understanding RPW's digestive system and its interaction with the environment [20]. Out of the three diets tested, coconuts were found to contain higher protein contents and were also the most preferred by RPW. The preference of RPW towards coconuts matched with the results of the protein profiles, with the major enzymes identified in the RPW's gut involved in the digestion of proteins being trypsin and aminopeptidase. The study discovered that the digestive enzyme matched with the dietary ingredient and a higher preference of the ingredient in the food for the larvae to digest [20].

Table 1. Summary of potentially targeted enzymes found in the gut of RPW and their functions.

Enzyme	EC Number	Function
Trypsin	3.4.21.4	Protein digestion
Aminopeptidase	3.4.11.1	Protein digestion
Xylanase	3.2.1.8	Xylan (plant cell wall) digestion
Glycosidase	3.2.1	Hydrolyze polysaccharide of cell wall
Amylase	3.2.1.1	Starch digestion
Catalase	1.11.1.6	Catalyze conversion of hydrogen peroxide into oxygen and water
Peroxidase	1.11.1.7	Catalyze conversion of hydrogen peroxide into oxygen and water
Polyphenol oxidase	1.14.18.1	Catalyze the oxidation of phenolic compound using oxygen

Other studies showed similar results matching digestive enzymes with food preferences. One study revealed that carbohydrate-metabolizing enzymes were more active in the gut of maize stem borer *Busseola fusca* [48] and fall armyworm *Spodoptera frugiperda* [49], as both insects had carbohydrates as their major food source. In addition, the protease

enzyme has also been characterized in the gut of RPW [50] and proven to be important as a target after it was tested for its inhibition with several insecticides in a separate study [51].

Apart from protein-metabolizing enzymes, the most common hemicellulase enzyme [52], xylanase, was found in the gut of RPW [53]. Xylanase (EC: 3.2.1.8) is an enzyme that hydrolyzes xylan (Table 1), which is the major constituent of hemicelluloses of plant cell walls. In addition, the carbohydrate-active enzyme called glycosidase, which is responsible for hydrolyzing complex carbohydrates and polysaccharides of plant cells into smaller products [54], and their protein family, namely amylase, have been identified and characterized in the gut of RPW [50,55–57]. Glycosidase (EC: 3.2.1), also referred to as glycoside hydrolase, is an enzyme that catalyzes the degradation of cell wall polysaccharides [58]. Amylase (EC: 3.2.1.1), which also belongs to a glucoside hydrolase family, is an enzyme that catalyzes the D-(1,4)-glucan linkage in starch and related carbohydrates in plant cells [55]. The existence of these enzymes is consistent with the behavior of RPW, which consume plant tissues. The silencing of the digestive enzyme, such as amylase [59] in RPW and xylanase [60] in coffee berry borer *Hypothenemus hampei* through injection of its respective dsRNA, was reported to cause mortality of the larvae.

In addition, the high activity of antioxidant enzymes (Table 1), such as catalase [61], polyphenols oxidase and peroxidase, involved in insect defense mechanisms were also detected and analyzed in the gut of RPW [62]. Antioxidant enzymes in insects are enzymes that can balance potentially harmful reactive oxygen species (ROS). ROS are produced due to changes in the biotic or abiotic factors, as well as exposure to insecticides [63]. Catalase (EC: 1.11.1.6) and peroxidase (EC: 1.11.1.7) function to catalyze the conversion of potentially toxic hydrogen peroxide into oxygen molecules and water [64]. Polyphenol oxidase (EC: 1.14.18.1) is an enzyme that uses oxygen to catalyze the oxidation of a wide range of phenolic compounds resulting from several factors, such as exposure to insecticides and starvation [62]. The functions of the antioxidant enzymes found in the gut of RPW showed that each of the enzymes could be targeted to control the pest. The enzymes were listed as some of the key enzymes of great potential to be targeted and knocked down using the RNAi approach [65]. It was proven that, when catalase was knocked down in an RNAi-based experiment, significant mortality and growth inhibition of RPW's larvae [66] and *Spodoptera litura* (Fabricius) larvae were recorded [67]. Moreover, the silencing of the catalase gene using the RNA interference approach in *Amblyomma maculatum* has resulted in a decrease in both egg mass and larval eclosion rates [68]. Furthermore, the knockdown of peroxidase [69] and polyphenol oxidase [70] in wheat aphid (*Sitobion avenae*) also led to a reduced survival rate and ecdysis index. Additionally, the RNAi-mediated silencing of salivary gland peroxidase in *Anopheles gambiae* caused a lower blood-feeding capacity [71], while the silencing of polyphenol oxidase in *Bombyx mori* caused incomplete pupation [72].

Most insects are highly dependent on the enzymes present in their gut for development and survival [73]. Therefore, analyzing the activity of the digestive and antioxidant enzymes in RPW's gut can contribute to the development of new insect pest control by preventing the digestion and assimilation of nutrients in the insect. Furthermore, the knockdown and silencing of potential digestive and antioxidant enzymes using RNAi is a promising and powerful approach to control and manage insect pests such as RPW [65].

5. RPW's Gut Transcriptome Analysis

RPW is a phytophagous pest that devours the wet woody trunk and very sugary sap of palm trees [74]. Similar to other herbivorous pests that feed on woody plants, these pests must detoxify the secondary metabolites, such as allelochemicals, produced by the plants [75]. This often leads to the development of metabolic adaptations that require various types of detoxifying enzymes. In some cases, the same enzymes may be responsible for both the pest's adaptation to natural plant defenses and its resistance to insecticides [76]. These metabolic adaptations may be the result of preexisting detoxifying enzymes within the pest's body, enzymes provided by microbial symbionts or enzymes acquired through horizontal gene transfer from fungi or bacteria [1].

There have been several reports stating the discovery of detoxifying genes (Table 2) in RPW's gut. Cytochrome P450 (CYP450) and glutathione-S-transferase (GST) were reported to be highly expressed in the mid-gut of RPW [62,75,77,78]. The detoxification system of insects consists of three phases: biotransforming, metabolizing and excreting. CYP450 was grouped into phase I, while GST was grouped into phase II [79]. CYP450 is a heme-containing protein that aids in the detoxification of insecticides by catalyzing the oxidation of the insecticide, leading to an increase in its solubility. This increase in solubility facilitates the removal of the insecticide from the body of the insect, resulting in its detoxification [77]. CYP450 can also work in conjunction with GST to detoxify insecticides. After the initial oxidation of the insecticide by CYP450, glutathione is added by GST to the oxidized compound to further enhance its elimination from the insect's body [1]. An RNAi experiment performed on CYP450 found in RPW showed an increasing insecticide susceptibility that led to RPW's death after it was tested with cypermethrin [4].

Table 2. Summary of potentially targeted genes/transcripts found in the gut of RPW and their functions.

Genes/Transcripts	EC Number	Function
Cytochrome P450	*	Catalyze oxidation of xenobiotics
glutathione-S-transferase	2.5.1.18	Added glutathione to oxidized xenobiotics
Laccase	1.10.3.2	Cuticle hardening
Carboxypeptidase	3.4.16.2	Protein digestion
Chitin synthase	2.4.1.16	Catalyzes the polymerization of chitin polymer
Cellulase	3.2.1.4	Cell wall degradation (cellulose)
Hemicellulase	3.2.1	Cell wall degradation (hemicellulose)
Pectinase	3.2.1.15	Cell wall degradation (pectin)
Neuropeptides	-	Trigger physiological process (digestion)

* Depends on the type of electron donor with which they interact [80]. - equals not available.

In addition, the transcript of a laccase enzyme (EC: 1.10.3.2) was found to be expressed in the gut of RPW (Table 2). This enzyme is a cuticular protein responsible for cuticle hardening to protect insects from environmental stress, and it may oxidize toxic compounds ingested by insects [81]. The injection of dsRNA into *Tribolium castaneum* designed for an RNAi experiment resulted in the depletion of laccase transcripts and caused the weevils to fail to tan, producing soft-bodied weevils that subsequently died [82,83].

Furthermore, a genome-wide analysis was conducted on digestion-related genes from the transcriptome data of female and male adult RPW. The analysis revealed 70 glycoside hydrolase genes, 17 α -amylase genes, 13 carboxypeptidase genes and 1 chitin synthase gene. The existence of carboxypeptidase (EC: 3.4.16.2) is important for stem borers such as RPW to catalyze the digestion of protein in the trunks of palm trees (Table 2) [84]. The significance of carboxypeptidase was demonstrated when the larvae of *Cosmopolites sordidus*, a banana weevil, showed lower survival rates and displayed a significant reduction of growth when it was inhibited by a soybean protease inhibitor [85].

On the other hand, chitin synthase (EC: 2.4.1.16) catalyzes the polymerization of the chitin polymer [86], a major component of insect cuticles, including the chitin layer that lines the gut of RPW (Table 2). This layer provides RPW with protection from rough food particles and ingested pathogens [1]. The inhibition of chitin synthase in cotton boll weevils (*Anthonomus grandis*) using RNAi recombinant transgenic cotton resulted in malformed first and third instar larvae. The report suggested that the knockdown of chitin synthase led to the obstruction of nutrient uptake in the gut [87].

In another study, the expression profiling of RPW's gut showed the identification of key enzymes in the digestion of plant cell walls (Table 2), namely cellulase (EC: 3.2.1.4), hemicellulase and pectinase (EC: 3.2.1.15) [54,88]. Those three enzymes function in catalyzing the degradation of major polysaccharides in the cell walls of most plant cells, including cellulose, hemicellulose and pectin [52]. As RPW is the major pest for many plant species by attacking and feeding on plant stems, the inhibition of these enzymes in RPW will lead to indigestion and starvation, eventually resulting in death [89].

Other than that, potentially targeted genes encoded for neuropeptide precursors and their receptors (Table 2) were found to be predominantly expressed in the gut of RPW. In insects, neuropeptides interact with their receptors and trigger signal transduction and physiological processes such as feeding and digestion [90]. A study found that an analogue insecticide targeting neuropeptides was applied to the insect pest *Myzus persicae*, resulting in a high mortality rate. This study also highlighted that the insecticide tested was not harmful towards beneficial insect *Chrysoperla carnea* [91].

Hence, the gut transcriptome analysis of RPW shows that it can offer valuable insights into cellular processes that occur in the gut. The identification of those genes and neuropeptides is necessary for the development of potential insecticides to effectively manage and prevent the invasion of RPW in palm trees [11,88,92].

6. *R. ferrugineus* Control Related to Its Digestive System

Having a comprehensive knowledge regarding the potential targets in the digestive system of RPW is helpful in developing and discovering new potential strategies in managing the RPW pest.

Although certain potential targets have been found to be responsive to insecticides or inhibitors [93], several other potential targets located in the gut of RPW have only been reported and not yet been specifically subjected to any insecticidal evaluation (Table 3). This creates a prospect for further research.

Table 3. Summary of tested insecticides and their potential targets in RPW's gut.

Insecticide/Inhibitor	Target Gene/Protein	Function of Target	Reference
<i>Thymus vulgaris</i> and <i>Ocimum basilicum</i> extract; soybean trypsin inhibitor; <i>N</i> -tosyl-L-phenylalanine chloromethyl ketone (chymotrypsin inhibitor)	Trypsin-like serine proteinase assessment; trypsin; chymotrypsin	Protein digestion	[50,51]
Hematoporphyrin dihydrochloride (photosensitizer)	Antioxidant enzymes (polyphenol oxidase; peroxidase)	Defense mechanism	[94]
Eserine (carbamate inhibitor)	Acetylcholinesterase (AChE)	Detoxifying enzyme	[16]
Protease inhibitor from palm dates kernel	Protease	Protein digestion	[95]
Novaluron	Chitinase	Chitin regulation	[18]
Sesquiterpene (Farnesol, Farnesyl acetate, Picrotoxin); Spinosad	Glutathione S transferase (GST), Cytochrome P450	Detoxification of xenobiotics	[77,96]
Spinosad	Nicotinic acetylcholine receptor and/or gamma Aminobutyric acid (GABA) receptor	Modulation of feeding behavior and reproduction	[96]
<i>Juniperus communis</i> essential oil	Gut protein content	Digestion system	[97]
RNAi/double strand RNA	Catalase	Defense mechanism	[61,66]
Aprotinin	Gut serine proteinase	Serine digestion	[98]
Protease inhibitor from <i>Vigna radiata</i> L. seeds	α -amylase	Carbohydrate digestion	[50,99]
Not tested	Neuropeptide precursor and receptor	To regulate physiology and behavior of insects	[90]
Not tested	Laccase	Oxidize toxic compounds ingested by the insect	[81]
Not tested	Xylanase	Digestion of plant cell wall	[53]
Not tested	Aminopeptidase	Protein digestion	[20]
Not tested	Cellulase	Digestion of plant cell wall	[88]
Not tested	Pectinase	Digestion of plant cell walls	[88,92]
Not tested	Glucosidase	Carbohydrate digestion	[56]

According to Zulkifli et al. [20], among the alternative methods in managing RPW infestations is targeting the potential proteins involved in nutrition or digestion. Identifying selective inhibitors of the digestive enzymes and potentially targeted proteins or genes in the RPW gut is important to turn off the enzymatic activity.

The discoveries of selective inhibitors, such as specific inhibitors targeting the trypsin and peptidase enzymes, which are crucial components of insecticides, can result in the disruption of digestion in the target insect. Consequently, this can impede the insect's growth and eventually lead to the mortality of the pest weevil. This corresponds with the findings by Mohamed et al. [53], who emphasized the importance of understanding the substances that inhibit digestive enzymes, such as the xylanolytic enzyme, as it can contribute to the development of control strategies for RPW infestations. An example of selective inhibitors is proteases inhibitors (PIs), which function as antimetabolic proteins that inhibit the insect's digestive activity. Orfali et al. [95] conducted research that demonstrated date kernel extract and *Calotropis latex* extract PIs were effective in inhibiting and reducing the activity of digestive protease enzymes in the gut of RPW by 39% and 18%, respectively, resulting in a decrease in the survival rate of the insect. This suggests that PIs obtained from plant extracts can serve as a valuable bioactive source for the development of biopesticides, essential for maintaining an ecological balance in the management of RPW insect pest infestations.

Another way of controlling RPW is by targeting the specific biosynthesis pathway involved in the digestive system of RPW. One of the potential target pathways for pest control in RPW is the chitin biosynthesis pathway [18]. As mentioned before, one of the transcripts found and suggested to be targeted is the chitin synthase (CHS2) gene, as it is involved in developing chitin in the gut lining of RPW. Chitin also builds up the well-developed mouthparts of adult and larvae RPW, which are strongly chitinized, enabling the pest to destroy the rough components of the palm vascular system [14]. It has been proven that chitin synthesis inhibitors such as chlorfluazuron, hexaflumuron and lufenuron showed efficiency against RPW larvae. Hence, the identification of chitin degradation-related transcripts from the digestive tract of RPW unfolds the RPW chitin degradation mechanism that might be manipulated for the development of targeted and specific future molecular insecticides [18].

7. Opportunity and Challenges of Targeting RPW's Digestive System

Studying RPW's digestive systems is crucial in agriculture, especially because of the detrimental impact of RPW as insect pests. With the advent of high-throughput analysis in metagenomics, proteomics and transcriptomics, the investigation of insects' digestive systems has progressed to the systems' biology level, encompassing the whole system rather than individual components. This approach offers insights into the dynamic nature of the digestive system and creates opportunities for further exploration [44].

The ability of phytophagous insects to survive is largely dependent on their capacity to effectively consume their respective host plant. As for RPW, its ability to consume and digest the host plant was reported to be aided by its insect-associated microbial communities in the gut [35,36]. Identifying and manipulating the microbial communities in the gut to manage the infestation of RPW is considered a novel management strategy [30]. However, the dynamic relationship between the symbiont's microbe communities and RPW remains poorly understood. As the diversity of the microbes can vary due to several factors, such as different host plants [37], temperature and pH [36], the dynamics need to be further investigated [35].

Additionally, gaining knowledge on the digestive enzymes that RPW produces when it infests different host plants can provide a way to learn how the insect picks a specific type of plant [48]. This knowledge can be used to prevent future infestations of RPW in new host plants. In addition, characterizing the digestive enzymes in the RPW gut offers an opportunity to be used in designing promising inhibitors and establishing effective pest management strategies [50]. However, some of the enzymes have only been biochemically

characterized and have not been tested with any inhibitor or insecticide yet. The proposed inhibitor or insecticide, however, needs to be designed as species-specific to avoid a leaching effect on nontarget organisms [100]. The symbiont-mediated RNAi (SMR) approach can be utilized to produce inhibitors for precise and specific pest management. This is an approach where symbiotic bacteria that have a limited host range were used to deliver insecticidal RNAi. This approach has been proposed to enhance the specificity of RNAi [101] and also been applied in Western flower thrips but not yet studied in RPW.

As part of RPW's digestive system is lined with chitin, targeting the chitin biosynthesis pathway is also an opportunity for controlling and managing the pest [18]. Chlorfluazuron, a chitin synthesis inhibitor reported to have a low and negligible leaching rate (<7%) [102], was proven to be effective on RPW [18]. However, chitin is one of the basic components that build the exoskeleton in the most insects. Therefore, the usage of a chitin inhibitor may affect not only RPW but also other beneficial insects such as bees [103]. However, this specificity towards nontarget organisms can be tackled by utilizing a docking analysis between several 3D structures of chitinase from the potentially affected nontarget organisms and the inhibitor. This analysis could be achieved using advanced computational approaches in combination with emerging artificial intelligence (AI) technologies, such as AlphaFold [104,105] and AutodockVina [106,107]. Hence, the structures of potentially affected nontarget organisms are urgently needed [103].

8. Conclusions

The review showed that there are many opportunities to explore and study the digestive system of RPW as a means for managing and controlling the infestation and spread of RPW as an insect pest. Metagenomic, proteomic and transcriptomic data were shown to be suitable approaches to further understanding the protein and gene constituents of RPW. However, with the advancement of technologies, the detection limit, the data generated and the tools for analysis no longer limit deeper exploration at the systemic level, opening the doors for gaining more knowledge on the digestive system of RPW. Hence, a more holistic approach could be used to produce a better way to manage RPW.

Author Contributions: Writing—Review and editing, A.-F.S.-K. and M.H.; writing—Original draft preparation, F.A.P. and A.N.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Higher Education (MOHE), Malaysia, through Long Term Research Grant Scheme (LRGS), under the grant number LRGS/F2/01/2020/08.

Data Availability Statement: No new data was created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Hazzouri, K.M.; Sudalaimuthuasari, N.; Kundu, B.; Nelson, D.; Al-Deeb, M.A.; Le Mansour, A.; Spencer, J.J.; Desplan, C.; Amiri, K.M.A. The Genome of Pest *Rhynchophorus ferrugineus* Reveals Gene Families Important at the Plant-Beetle Interface. *Commun. Biol.* **2020**, *3*, 323. [[CrossRef](#)]
2. Azmi, W.A.; Lian, C.J.; Zakeri, H.A.; Yusuf, N.; Omar, W.B.W.; Wai, Y.K.; Zulkefli, A.N.; Hussain, M.H. The Red Palm Weevil, *Rhynchophorus ferrugineus*: Current Issues and Challenges in Malaysia. *Oil Palm. Bull.* **2017**, *74*, 17–24.
3. Harith-Fadzilah, N.; Haris-Hussain, M.; Abd Ghani, I.; Zakaria, A.; Amit, S.; Zainal, Z.; Azmi, W.A.; Jalinas, J.; Hassan, M. Physical and Physiological Monitoring on Red Palm Weevil-Infested Oil Palms. *Insects* **2020**, *11*, 407. [[CrossRef](#)]
4. Ahmad, J.N.; Manzoor, M.; Aslam, Z.; Ahmad, S.J.N. Molecular Study on Field Evolved Resistance of Red Palm Weevil (*Rhynchophorus Ferruginous*) and Its Management through RNAi. *PJZ* **2020**, *52*, 477. [[CrossRef](#)]
5. Siriwardena, K.A.P.; Fernando, L.C.P.; Nanayakkara, N.; Perera, K.F.G.; Kumara, A.D.N.T.; Nanayakkara, T. Portable Acoustic Device for Detection of Coconut Palms Infested by *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *Crop. Prot.* **2010**, *29*, 25–29. [[CrossRef](#)]

6. Hussain, M.H. Efficacy of Baits for Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* Olivier under Constant Laboratory Condition. *JOPR* **2020**, *32*, 355–364. [[CrossRef](#)]
7. Soroker, V.; Colazza, S. *Handbook of Major Palm Pests: Biology and Management*; John Wiley & Sons: Hoboken, NJ, USA, 2017; ISBN 978-1-119-05745-1.
8. Leatemia, J.A.; Patty, J.A.; Masauna, E.D.; Noya, S.H.; Hasinu, J.V. Utilization of Sago Grub (*Rhynchophorus ferrugineus* Olivier) (Coleoptera: Curculionidae) as an Alternative Source of Protein. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *800*, 012028. [[CrossRef](#)]
9. El-Mergawy, R.; Al-Ajlan, A.M. Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier): Economic Importance, Biology, Biogeography and Integrated Pest Management. *J. Agric. Sci. Technol. A* **2011**, *1*, 1–23.
10. Nurashikin-Khairuddin, W.; Abdul-Hamid, S.N.A.; Mansor, M.S.; Bharudin, I.; Othman, Z.; Jalinias, J. A Review of Entomopathogenic Nematodes as a Biological Control Agent for Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *Insects* **2022**, *13*, 245. [[CrossRef](#)]
11. Yang, H.; Xu, D.; Zhuo, Z.; Hu, J.; Lu, B. Transcriptome and Gene Expression Analysis of *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) during Developmental Stages. *PeerJ* **2020**, *8*, e10223. [[CrossRef](#)]
12. Harris, M.N.; Norzainih, J.J.; Nurul Wahida, O. Morphology and Histology of the Digestive System of the Red Palm Weevil Larva, *Rhynchophorus ferrugineus*, Olivier (Coleoptera: Dryophthoridae). In Proceedings of the 3rd International Conference on Chemical, Agricultural and Medical Sciences (CAMS-2015), Singapore, 10–11 December 2015; International Institute of Chemical, Biological & Environmental Engineering: Phuket, Thailand, 2015; pp. 32–37.
13. El-Fattah, A.Y.A.; El-Wahab, A.S.A.; Jamal, Z.A.; El-Helaly, A.A. Histopathological Studies of Red Palm Weevil *Rhynchophorus ferrugineus*, (Olivier) Larvae and Adults to Evaluate Certain Nano Pesticides. *Braz. J. Biol.* **2021**, *81*, 195–201. [[CrossRef](#)]
14. El-Sobki, A.; Ali, A. Biochemical Effects of Some Chitin Synthesis Inhibitors Against Red Palm Weevil, *Rhynchophorus ferrugineus* Insect. *Egypt. Acad. J. Biol. Sci. F. Toxicol. Pest Control* **2020**, *12*, 127–139. [[CrossRef](#)]
15. Harris, M.N.; Shafinaz, N.; Yaakop, S.; Othman, N.W. Distribution of Serotonin (5-HT) and Dopamine (DA) on Digestive Tract of Red Palm Weevil Larva, *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae). *Sains Malays* **2016**, *21*, 39–50.
16. Mohamed, M.A.; Shaalan, S.; Ghazy, A.-E.M.; Ali, A.A.; Abd-Elaziz, A.M.; Ghanem, M.M.E.; Abd-Elghany, S.A. Purification and Characterization of Acetylcholinesterase in *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae). *Int. J. Biol. Macromol.* **2020**, *147*, 1029–1040. [[CrossRef](#)]
17. Wakil, W.; Yasin, M.; Qayyum, M.A.; Ghazanfar, M.U.; Al-Sadi, A.M.; Bedford, G.O.; Kwon, Y.J. Resistance to Commonly Used Insecticides and Phosphine Fumigant in Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) in Pakistan. *PLoS ONE* **2018**, *13*, e0192628. [[CrossRef](#)]
18. Hussain, A.; AlJabr, A.M.; Al-Ayedh, H. Development-Disrupting Chitin Synthesis Inhibitor, Novaluron, Reprogramming the Chitin Degradation Mechanism of Red Palm Weevils. *Molecules* **2019**, *24*, 4304. [[CrossRef](#)]
19. Mahadi, N.A.; Yusof, T.A.A.; Mat, M.; Abdullah, A.; Zakaria, M.H.; Masdor, N.A. Comparative Study of Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) Reproduction Reared Using Different Diets. *Am. J. Entomol.* **2022**, *6*, 110–115. [[CrossRef](#)]
20. Zulkifli, A.N.; Zakeri, H.A.; Azmi, W.A. Food Consumption, Developmental Time, and Protein Profile of the Digestive System of the Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) Larvae Reared on Three Different Diets. *J. Insect Sci.* **2018**, *18*, 10. [[CrossRef](#)]
21. Haldhar, S.M.; Muralidharan, C.M.; Singh, D. Pests and Their Management in Date Palm. In *Trends in Horticultural Entomology*; Springer: Singapore, 2022; pp. 833–845.
22. Dembilio, Ó.; Jaques, J.A. Biology and Management of Red Palm Weevil. In *Sustainable Pest Management in Date Palm: Current Status and Emerging Challenges*; Wakil, W., Romeno Faleiro, J., Miller, T.A., Eds.; Sustainability in Plant and Crop Protection; Springer International Publishing: Cham, Switzerland, 2015; pp. 13–36. ISBN 978-3-319-24397-9.
23. Martin, B. A Relative Analysis on Sound of Red Palm Weevil Based on Field and Lab Recordings. *Int. J. Appl. Eng. Res.* **2015**, *10*, 5261–5268.
24. Muhammad, A.; Habineza, P.; Hou, Y.; Shi, Z. Preparation of Red Palm Weevil *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Dryophthoridae) Germ-Free Larvae for Host-Gut Microbes Interaction Studies. *BIO-PROTOCOL* **2019**, *9*, e3456. [[CrossRef](#)]
25. Jing, T.-Z.; Qi, F.-H.; Wang, Z.-Y. Most Dominant Roles of Insect Gut Bacteria: Digestion, Detoxification, or Essential Nutrient Provision? *Microbiome* **2020**, *8*, 38. [[CrossRef](#)] [[PubMed](#)]
26. Yun, J.-H.; Roh, S.W.; Whon, T.W.; Jung, M.-J.; Kim, M.-S.; Park, D.-S.; Yoon, C.; Nam, Y.-D.; Kim, Y.-J.; Choi, J.-H.; et al. Insect Gut Bacterial Diversity Determined by Environmental Habitat, Diet, Developmental Stage, and Phylogeny of Host. *Appl. Environ. Microbiol.* **2014**, *80*, 5254–5264. [[CrossRef](#)]
27. Engel, P.; Moran, N.A. The Gut Microbiota of Insects—Diversity in Structure and Function. *FEMS Microbiol. Rev.* **2013**, *37*, 699–735. [[CrossRef](#)] [[PubMed](#)]
28. Farah Nadiyah, R.; Norefrina Shafinaz, M.N.; Nurul Wahida, O. Preliminary Study of Gut Bacterial Abundance in *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) Fed on Different Diets. *Serangga* **2018**, *23*, 126–138.
29. Mason, C.J.; St. Clair, A.; Peiffer, M.; Gomez, E.; Jones, A.G.; Felton, G.W.; Hoover, K. Diet Influences Proliferation and Stability of Gut Bacterial Populations in Herbivorous Lepidopteran Larvae. *PLoS ONE* **2020**, *15*, e0229848. [[CrossRef](#)]

30. Liu, Q.-X.; Su, Z.-P.; Liu, H.-H.; Lu, S.-P.; Ma, B.; Zhao, Y.; Hou, Y.-M.; Shi, Z.-H. The Effect of Gut Bacteria on the Physiology of Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier and Their Potential for the Control of This Pest. *Insects* **2021**, *12*, 594. [[CrossRef](#)] [[PubMed](#)]
31. Muhammad, A.; Habineza, P.; Ji, T.; Hou, Y.; Shi, Z. Intestinal Microbiota Confer Protection by Priming the Immune System of Red Palm Weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Dryophthoridae). *Front. Physiol.* **2019**, *10*, 1303. [[CrossRef](#)]
32. Anbutsu, H.; Moriyama, M.; Nikoh, N.; Hosokawa, T.; Futahashi, R.; Tanahashi, M.; Meng, X.-Y.; Kuriwada, T.; Mori, N.; Oshima, K. Small Genome Symbiont Underlies Cuticle Hardness in Beetles. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, E8382–E8391. [[CrossRef](#)]
33. Habineza, P.; Muhammad, A.; Ji, T.; Xiao, R.; Yin, X.; Hou, Y.; Shi, Z. The Promoting Effect of Gut Microbiota on Growth and Development of Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Dryophthoridae) by Modulating Its Nutritional Metabolism. *Front. Microbiol.* **2019**, *10*, 1212. [[CrossRef](#)]
34. Manee, M.M.; Alqahtani, F.H.; Al-Shomrani, B.M.; El-Shafie, H.A.F.; Dias, G.B. Omics in the Red Palm Weevil *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae): A Bridge to the Pest. *Insects* **2023**, *14*, 255. [[CrossRef](#)]
35. Muhammad, A.; Fang, Y.; Hou, Y.; Shi, Z. The Gut Entomotype of Red Palm Weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Dryophthoridae) and Their Effect on Host Nutrition Metabolism. *Front. Microbiol.* **2017**, *8*, 2291. [[CrossRef](#)]
36. Jia, S.; Zhang, X.; Zhang, G.; Yin, A.; Zhang, S.; Li, F.; Wang, L.; Zhao, D.; Yun, Q.; Tala; et al. Seasonally Variable Intestinal Metagenomes of the Red Palm Weevil (*Rhynchophorus ferrugineus*). *Environ. Microbiol.* **2013**, *15*, 3020–3029. [[CrossRef](#)] [[PubMed](#)]
37. Wei, J.; Yang, X.-K.; Zhang, S.-K.; Seagraves, K.A.; Xue, H.-J. Parallel Metatranscriptome Analysis Reveals Degradation of Plant Secondary Metabolites by Beetles and Their Gut Symbionts. *Mol. Ecol.* **2022**, *31*, 3999–4016. [[CrossRef](#)]
38. Yaman, M.; Ertürk, Ö.; Aslan, İ. Isolation of Some Pathogenic Bacteria from the Great Spruce Bark Beetle, *Dendroctonus micans* and Its Specific Predator, *Rhizophagus grandis*. *Folia Microbiol.* **2010**, *55*, 35–38. [[CrossRef](#)]
39. Anand, A.A.P.; Vennison, S.J.; Sankar, S.G.; Prabhu, D.I.G.; Vasan, P.T.; Raghuraman, T.; Geoffrey, C.J.; Vendan, S.E. Isolation and Characterization of Bacteria from the Gut of *Bombyx mori* that Degrade Cellulose, Xylan, Pectin and Starch and Their Impact on Digestion. *J. Insect Sci.* **2010**, *10*, 107. [[CrossRef](#)]
40. Jancek, S.; Bézier, A.; Gayral, P.; Paillusson, C.; Kaiser, L.; Dupas, S.; Le Ru, B.P.; Barbe, V.; Periquet, G.; Drezen, J.-M.; et al. Adaptive Selection on Bracovirus Genomes Drives the Specialization of Cotesia Parasitoid Wasps. *PLoS ONE* **2013**, *8*, e64432. [[CrossRef](#)] [[PubMed](#)]
41. Gopinadhan, P.B.; Mohandas, N.; Vasudevan Nair, K.P. Cytoplasmic Polyhedrosis Virus Infecting Redpalm Weevil of Coconut. *Curr. Sci. Assoc.* **1990**, *59*, 577–580.
42. Banerjee, A.; Dangar, T.K. *Pseudomonas aeruginosa*, a Facultative Pathogen of Red Palm Weevil, *Rhynchophorus ferrugineus*. *World J. Microbiol. Biotechnol.* **1995**, *11*, 618–620. [[CrossRef](#)] [[PubMed](#)]
43. Salama, H.S.; Foda, M.S.; El-Bendary, M.A.; Abdel-Razek, A. Infection of Red Palm Weevil, *Rhynchophorus ferrugineus*, by Spore-Forming Bacilli Indigenous to Its Natural Habitat in Egypt. *J. Pest Sci.* **2004**, *77*, 27–31. [[CrossRef](#)]
44. Muñoz-Benavent, M.; Pérez-Cobas, A.E.; García-Ferris, C.; Moya, A.; Latorre, A. Insects' Potential: Understanding the Functional Role of Their Gut Microbiome. *J. Pharm. Biomed. Anal.* **2021**, *194*, 113787. [[CrossRef](#)]
45. Wang, J.-M.; Bai, J.; Zheng, F.-Y.; Ling, Y.; Li, X.; Wang, J.; Zhi, Y.-C.; Li, X.-J. Diversity of the Gut Microbiome in Three Grasshopper Species Using 16S rRNA and Determination of Cellulose Digestibility. *PeerJ* **2020**, *8*, e10194. [[CrossRef](#)] [[PubMed](#)]
46. Dantur, K.I.; Enrique, R.; Welin, B.; Castagnaro, A.P. Isolation of Cellulolytic Bacteria from the Intestine of *Diatraea saccharalis* Larvae and Evaluation of Their Capacity to Degrade Sugarcane Biomass. *AMB Expr.* **2015**, *5*, 15. [[CrossRef](#)]
47. Zhang, X.; Zhang, F.; Lu, X. Diversity and Functional Roles of the Gut Microbiota in Lepidopteran Insects. *Microorganisms* **2022**, *10*, 1234. [[CrossRef](#)] [[PubMed](#)]
48. Juma, G.; Le Ru, B.; Calatayud, P.-A. Assortments of Digestive Enzymes Induced in First Instar Larvae of *Busseola fusca* Feeding on Different Plants. *Int. J. Insect Sci.* **2019**, *11*, 117954331984352. [[CrossRef](#)] [[PubMed](#)]
49. Lwalaba, D.; Hoffmann, K.H.; Woodring, J. Control of the Release of Digestive Enzymes in the Larvae of the Fall Armyworm, *Spodoptera frugiperda*. *Arch. Insect Biochem. Physiol.* **2010**, *73*, 14–29. [[CrossRef](#)] [[PubMed](#)]
50. Abd El-latif, A.O. Partial Characterization of the Digestive Proteases and α -Amylase of the Larvae of the Red Palm Weevil, *Rhynchophorus ferrugineus*. *Arthropods* **2020**, *9*, 7–14.
51. Darrag, H.M.; Almuhan, H.T.; Hakami, E.H.; Alhojaily, S.M. Analysis of Volatile Secondary Metabolites in *Ocimum basilicum* Cell Suspensions: Inhibition, In Silico Molecular Docking, and an ADMET Analysis against Proteolytic Enzymes of *Rhynchophorus ferrugineus*. *Plants* **2022**, *11*, 2949. [[CrossRef](#)]
52. Gao, P.; Liu, Z.; Wen, J. Expression Profiling of Plant Cell Wall-Degrading Enzyme Genes in *Eucryptorrhynchus scrobiculatus* Midgut. *Front. Physiol.* **2020**, *11*, 1111. [[CrossRef](#)] [[PubMed](#)]
53. Mohamed, M.A.; Ghanem, M.M.E.; Abd-Elaziz, A.M.; Shams-Eldin, I.M. Purification and Characterization of Xylanase Isoenzymes from Red Palm Weevil *Rhynchophorus ferrugineus*. *Biocatal. Agric. Biotechnol.* **2018**, *14*, 321–327. [[CrossRef](#)]
54. Rafiei, V.; Vélèz, H.; Tzelepis, G. The Role of Glycoside Hydrolases in Phytopathogenic Fungi and Oomycetes Virulence. *IJMS* **2021**, *22*, 9359. [[CrossRef](#)]
55. Darvishzadeh, A.; Bandani, A.R.; Karimi, J.; Timouri, G. Biochemical Characterisation of Digestive α -Amylase of Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Curculionidae). *Arch. Phytopathol. Plant Prot.* **2012**, *45*, 2132–2142. [[CrossRef](#)]

56. Darvishzadeh, A.; Bandani, A.R. Identification and Enzymatic Characterisation of Digestive Glucosidases from Gut of Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Curculionidae). *Arch. Phytopathol. Plant Prot.* **2013**, *46*, 1159–1167. [[CrossRef](#)]
57. Riseh, N.S.; Ghadamyari, M.; Motamediniya, B. Biochemical Characterisation of α - and β -Glucosidases and α - and β -Galactosidases from Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) (Col.: Curculionide). *Plant Prot. Sci.* **2012**, *48*, 85–93. [[CrossRef](#)]
58. Dong, X.; Jiang, Y.; Hur, Y. Genome-Wide Analysis of Glycoside Hydrolase Family 1 β -Glucosidase Genes in Brassica Rapa and Their Potential Role in Pollen Development. *IJMS* **2019**, *20*, 1663. [[CrossRef](#)] [[PubMed](#)]
59. Laudani, F.; Strano, C.P.; Edwards, M.G.; Malacrino, A.; Campolo, O.; Halim, H.M.A.E.; Gatehouse, A.M.R.; Palmeri, V. RNAi-Mediated Gene Silencing in *Rhynchophorus ferrugineus* (Oliver) (Coleoptera: Curculionidae). *Open Life Sci.* **2017**, *12*, 214–222. [[CrossRef](#)]
60. Aguilera, C.; Padilla, B.E.; Flórez, C.P.; Rubio, J.D.; Acuña, J.R. RNA interference: Potential uses on functional genomics and genetic control of *Hypothenemus hampei* (Coleoptera: Scolytinae). *Rev. Colomb. Entomol.* **2011**, *37*, 167–172. [[CrossRef](#)]
61. Ghanem, M.M.E.; Mohamed, M.A.; Abd-Elaziz, A.M. Distribution, Purification and Characterization of a Monofunctional Catalase from *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae). *Biocatal. Agric. Biotechnol.* **2020**, *23*, 101480. [[CrossRef](#)]
62. Mohamed, M.A.; Ghazy, A.-E.M.; Abdel Karim, G.S.A.; El-khonezy, M.I.; Abd-Elaziz, A.M.; Ghanem, M.M.E. Defense Status in Larval Stage of Red Palm Weevil *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae). *Biocatal. Agric. Biotechnol.* **2022**, *44*, 102465. [[CrossRef](#)]
63. Kaur, M.; Chadha, P.; Kaur, S.; Kaur, A. Effect of *Aspergillus Flavus* on Lipid Peroxidation and Activity of Antioxidant Enzymes in Midgut Tissue of *Spodoptera litura* Larvae. *Arch. Phytopathol. Plant Prot.* **2021**, *54*, 177–190. [[CrossRef](#)]
64. Sandhu, R.K.; Sarao, P.S.; Sharma, N. Antibiosis in Wild Rice Accessions Induced by *Nilaparvata lugens* (Stål) Feeding. *Phytoparasitica* **2020**, *48*, 801–812. [[CrossRef](#)]
65. Kola, V.S.R.; Renuka, P.; Madhav, M.S.; Mangrauthia, S.K. Key Enzymes and Proteins of Crop Insects as Candidate for RNAi Based Gene Silencing. *Front. Physiol.* **2015**, *6*, 119. [[CrossRef](#)] [[PubMed](#)]
66. Al-Ayedh, H.; Rizwan-ul-Haq, M.; Hussain, A.; Aljabr, A.M. Insecticidal Potency of RNAi-Based Catalase Knockdown in *Rhynchophorus ferrugineus* (Oliver) (Coleoptera: Curculionidae): Catalase Knockdown in *Rhynchophorus ferrugineus*. *Pest. Manag. Sci.* **2016**, *72*, 2118–2127. [[CrossRef](#)] [[PubMed](#)]
67. Zhao, H.; Yi, X.; Hu, Z.; Hu, M.; Chen, S.; Muhammad, R.-H.; Dong, X.; Gong, L. RNAi-Mediated Knockdown of Catalase Causes Cell Cycle Arrest in SL-1 Cells and Results in Low Survival Rate of *Spodoptera litura* (Fabricius). *PLoS ONE* **2013**, *8*, e59527. [[CrossRef](#)] [[PubMed](#)]
68. Kumar, D.; Budachetri, K.; Meyers, V.C.; Karim, S. Assessment of Tick Antioxidant Responses to Exogenous Oxidative Stressors and Insight into the Role of Catalase in the Reproductive Fitness of the Gulf Coast Tick, *Amblyomma maculatum*: Antioxidant Responses in Gulf Coast Ticks. *Insect Mol. Biol.* **2016**, *25*, 283–294. [[CrossRef](#)]
69. Deng, F.; He, Q.; Zhao, Z. Suppressing a Peroxidase Gene Reduces Survival in the Wheat Aphid *Sitobion avenae*. *Arch. Insect Biochem. Physiol.* **2016**, *93*, 86–95. [[CrossRef](#)] [[PubMed](#)]
70. Zhang, Y.; Fan, J.; Francis, F.; Chen, J. Molecular Characterization and Gene Silencing of *Laccase 1* in the Grain Aphid, *Sitobion avenae*. *Arch. Insect Biochem. Physiol.* **2018**, *97*, e21446. [[CrossRef](#)]
71. Das, S.; Radtke, A.; Choi, Y.-J.; Mendes, A.M.; Valenzuela, J.G.; Dimopoulos, G. Transcriptomic and Functional Analysis of the Anopheles Gambiae Salivary Gland in Relation to Blood Feeding. *BMC Genom.* **2010**, *11*, 566. [[CrossRef](#)]
72. Wang, M.; Lu, Y.; Cai, Z.; Liang, S.; Niu, Y.; Miao, Y. Phenol Oxidase Is a Necessary Enzyme for the Silkworm Molting Which Is Regulated by Molting Hormone. *Mol. Biol. Rep.* **2013**, *40*, 3549–3555. [[CrossRef](#)]
73. Wu, K.; Li, S.; Wang, J.; Ni, Y.; Huang, W.; Liu, Q.; Ling, E. Peptide Hormones in the Insect Midgut. *Front. Physiol.* **2020**, *11*, 191. [[CrossRef](#)]
74. Khudri, N.A.F.R.S.; Mohd Masri, M.M.; Maidin, M.S.T.; Kamarudin, N.; Hussain, M.H.; Abd Ghani, I.; Jalinas, J. Preliminary Evaluation of Acoustic Sensors for Early Detection of Red Palm Weevil, *Rhynchophorus ferrugineus* Incidence on Oil Palm and Coconut in Malaysia. *Int. J. Trop. Insect Sci.* **2021**, *41*, 3287–3292. [[CrossRef](#)]
75. AlJabr, A.; Hussain, A.; Rizwan-ul-Haq, M.; Al-Ayedh, H. Toxicity of Plant Secondary Metabolites Modulating Detoxification Genes Expression for Natural Red Palm Weevil Pesticide Development. *Molecules* **2017**, *22*, 169. [[CrossRef](#)]
76. Jaffar, S.; Ahmad, S.; Lu, Y. Contribution of Insect Gut Microbiota and Their Associated Enzymes in Insect Physiology and Biodegradation of Pesticides. *Front. Microbiol.* **2022**, *13*, 979383. [[CrossRef](#)] [[PubMed](#)]
77. Hussain, A.; Rizwan-ul-haq, M.; AlJabr, A.M.; Al-Ayedh, H. Lethality of Sesquiterpenes Reprogramming Red Palm Weevil Detoxification Mechanism for Natural Novel Biopesticide Development. *Molecules* **2019**, *24*, 1648. [[CrossRef](#)] [[PubMed](#)]
78. Manzoor, M.; Yang, L.; Wu, S.; El-Shafie, H.; Haider, M.S.; Ahmad, J.N. Feeding Preference of *Rhynchophorus ferrugineus* (Oliver) (Coleoptera: Curculionidae) on Different Date Palm Cultivars and Host Biochemical Responses to Its Infestation. *Bull. Entomol. Res.* **2022**, *112*, 494–501. [[CrossRef](#)]
79. Hu, B.; Zhang, S.; Ren, M.; Tian, X.; Wei, Q.; Mburu, D.K.; Su, J. The Expression of *Spodoptera exigua* P450 and UGT Genes: Tissue Specificity and Response to Insecticides. *Insect Sci.* **2019**, *26*, 199–216. [[CrossRef](#)] [[PubMed](#)]

80. Pandian, B.A.; Sathishraj, R.; Djanaguiraman, M.; Prasad, P.V.V.; Jugulam, M. Role of Cytochrome P450 Enzymes in Plant Stress Response. *Antioxidants* **2020**, *9*, 454. [[CrossRef](#)]
81. Babiker, M.A.A.-B.; Hamadttu, A.F.E.-S. Expression Profiling, Phylogenetic, and Structural Analyses of a Laccase Gene from the Red Palm Weevil, *Rhynchophorus ferrugineus*. *Afr. J. Biotechnol.* **2019**, *18*, 978–990. [[CrossRef](#)]
82. Arakane, Y.; Muthukrishnan, S.; Beeman, R.W.; Kanost, M.R.; Kramer, K.J. Laccase 2 Is the Phenoloxidase Gene Required for Beetle Cuticle Tanning. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 11337–11342. [[CrossRef](#)]
83. Dittmer, N.T.; Gorman, M.J.; Kanost, M.R. Characterization of Endogenous and Recombinant Forms of Laccase-2, a Multicopper Oxidase from the Tobacco Hornworm, *Manduca sexta*. *Insect Biochem. Mol. Biol.* **2009**, *39*, 596–606. [[CrossRef](#)]
84. Ben Thabet, I.; Francis, F.; De Pauw, E.; Besbes, S.; Attia, H.; Deroanne, C.; Blecker, C. Characterisation of Proteins from Date Palm Sap (*Phoenix dactylifera* L.) by a Proteomic Approach. *Food Chem.* **2010**, *123*, 765–770. [[CrossRef](#)]
85. Montesdeoca, M.; Lobo, M.G.; Casanas, N.; Carnero, A.; Castanera, P.; Ortego, F. Partial Characterization of the Proteolytic Enzymes in the Gut of the Banana Weevil, *Cosmopolites sordidus*, and Effects of Soybean Kunitz Trypsin Inhibitor on Larval Performance. *Entomol. Exp. Appl.* **2005**, *116*, 227–236. [[CrossRef](#)]
86. Ranganathan, S.; Ampasala, D.R.; Palaka, B.K.; Ilavarasi, A.V.; Patidar, I.; Poovadan, L.P.; Sapam, T.D. In Silico Binding Profile Analysis and In Vitro Investigation on Chitin Synthase Substrate and Inhibitors from Maize Stem Borer, *Chilo partellus*. *Curr. Comput.-Aided Drug Des.* **2021**, *17*, 881–895. [[CrossRef](#)]
87. Ribeiro, T.P.; Vasquez, D.D.N.; Macedo, L.L.P.; Lourenço-Tessutti, I.T.; Valença, D.C.; Oliveira-Neto, O.B.; Paes-de-Melo, B.; Rodrigues-Silva, P.L.; Firmino, A.A.P.; Basso, M.F.; et al. Stabilized Double-Stranded RNA Strategy Improves Cotton Resistance to CBW (*Anthonomus grandis*). *Int. J. Mol. Sci.* **2022**, *23*, 13713. [[CrossRef](#)] [[PubMed](#)]
88. Antony, B.; Johny, J.; Aldosari, S.A.; Abdelazim, M.M. Identification and Expression Profiling of Novel Plant Cell Wall Degrading Enzymes from a Destructive Pest of Palm Trees, *Rhynchophorus ferrugineus*: Red Palm Weevil Plant Cell Wall Degrading Enzymes. *Insect Mol. Biol.* **2017**, *26*, 469–484. [[CrossRef](#)] [[PubMed](#)]
89. Abdel-Hameid, N.F. Starvation Effect on Bioactive Components Ofthe Red Palm Weevil *Rhynchophorus ferrugineus* (Olivier); (Coleoptera: Curculionidae). *Sciences* **2018**, *8*, 337–344.
90. Zhang, H.; Bai, J.; Huang, S.; Liu, H.; Lin, J.; Hou, Y. Neuropeptides and G-Protein Coupled Receptors (GPCRs) in the Red Palm Weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Dryophthoridae). *Front. Physiol.* **2020**, *11*, 159. [[CrossRef](#)]
91. Shi, Y.; Pandit, A.; Nachman, R.J.; Christiaens, O.; Davies, S.A.; Dow, J.A.T.; Smagghe, G. Transcriptome Analysis of Neuropeptides in the Beneficial Insect Lacewing (*Chrysoperla carnea*) Identifies Kinins as a Selective Pesticide Target: A Biostable Kinin Analogue with Activity against the Peach Potato Aphid *Myzus persicae*. *J. Pest Sci.* **2023**, *96*, 253–264. [[CrossRef](#)]
92. Vatanparast, M.; Hosseininaveh, V.; Ghadamyari, M.; Minoo Sajjadian, S. Plant Cell Wall Degrading Enzymes, Pectinase and Cellulase, in the Digestive System of the Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *Plant Prot. Sci.* **2014**, *50*, 190–198. [[CrossRef](#)]
93. Siddiqui, J.A.; Khan, M.M.; Bamisile, B.S.; Hafeez, M.; Qasim, M.; Rasheed, M.T.; Rasheed, M.A.; Ahmad, S.; Shahid, M.I.; Xu, Y. Role of Insect Gut Microbiota in Pesticide Degradation: A Review. *Front. Microbiol.* **2022**, *13*, 870462. [[CrossRef](#)]
94. Abdel-Moaty, Z.; Abdelsalam, S. Photosensitizing Effects of Hematoporphyrin Dihydrochloride against the Red Palm Weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *Fresenius Environ. Bull.* **2021**, *30*, 8158.
95. Orfali, R.; Binsuwaileh, A.; Abu Al-Ala'a, H.; Bane-Gamea, S.; Zaidan, N.; Abdelazim, M.; Alhasan Ismael, M.; Perveen, S.; Majrashi, N.; Alluhayb, K.; et al. Production of a Biopesticide on Host and Non-Host Serine Protease Inhibitors for Red Palm Weevil in Palm Trees. *Saudi J. Biol. Sci.* **2020**, *27*, 2803–2808. [[CrossRef](#)]
96. Abdelsalam, S.A.; Alzahrani, A.M.; Elmenshawy, O.M.; Abdel-Moneim, A.M. Spinosad Induces Antioxidative Response and Ultrastructure Changes in Males of Red Palm Weevil *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae). *J. Insect Sci.* **2016**, *16*, 106. [[CrossRef](#)] [[PubMed](#)]
97. Aziza, S.; Mona, E.-D. Toxic and Biochemical Effects of *Juniperus communis* Essential Oil on the Red Palm Weevil *Rhynchophorus ferrugineus* (Olivier.) (Coleoptera: Curculionidae). *Egypt. J. Biol. Pest Control* **2016**, *26*, 339.
98. Josephrajakumar, A.; Mohan, C.; Chaturvedi, V.K. Suppression of Growth and Endopeptidases of Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) Infesting Coconut Using Proteinase Inhibitors. *Entomon* **2016**, *41*, 283–292.
99. Riseh, N.S.; Ghadamyari, M. Biochemical Characterization of α -Amylases from Gut and Hemolymph of *Rhynchophorus ferrugineus* Olivieri (Col.: Curculionidae) and Their Inhibition by Extracts from the Legumes *Vigna radiata* L. and *Phaseolus vulgaris* L. *Invertebr. Surviv. J.* **2012**, *9*, 72–81.
100. Lu, Y.; Deng, X.; Zhu, Q.; Wu, D.; Zhong, J.; Wen, L.; Yu, X. The DsRNA Delivery, Targeting and Application in Pest Control. *Agronomy* **2023**, *13*, 714. [[CrossRef](#)]
101. Whitten, M.M.A.; Xue, Q.; Taning, C.N.T.; James, R.; Smagghe, G.; Del Sol, R.; Hitchings, M.; Dyson, P. A Narrow Host-Range and Lack of Persistence in Two Non-Target Insect Species of a Bacterial Symbiont Exploited to Deliver Insecticidal RNAi in Western Flower Thrips. *Front. Insect Sci.* **2023**, *3*, 1093970. [[CrossRef](#)]
102. Li, H.; Zhong, Q.; Wang, X.; Luo, F.; Zhou, L.; Sun, H.; Yang, M.; Lou, Z.; Chen, Z.; Zhang, X. The Degradation and Metabolism of Chlorfluazuron and Flonicamid in Tea: A Risk Assessment from Tea Garden to Cup. *Sci. Total Environ.* **2021**, *754*, 142070. [[CrossRef](#)]
103. Chen, W.; Yang, Q. Development of Novel Pesticides Targeting Insect Chitinases: A Minireview and Perspective. *J. Agric. Food Chem.* **2020**, *68*, 4559–4565. [[CrossRef](#)]

104. Jumper, J.; Evans, R.; Pritzel, A.; Green, T.; Figurnov, M.; Ronneberger, O.; Tunyasuvunakool, K.; Bates, R.; Žídek, A.; Potapenko, A.; et al. Highly Accurate Protein Structure Prediction with AlphaFold. *Nature* **2021**, *596*, 583–589. [[CrossRef](#)]
105. Varadi, M.; Anyango, S.; Deshpande, M.; Nair, S.; Natassia, C.; Yordanova, G.; Yuan, D.; Stroe, O.; Wood, G.; Laydon, A.; et al. AlphaFold Protein Structure Database: Massively Expanding the Structural Coverage of Protein-Sequence Space with High-Accuracy Models. *Nucleic Acids Res.* **2022**, *50*, D439–D444. [[CrossRef](#)] [[PubMed](#)]
106. Trott, O.; Olson, A.J. AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. *J. Comput. Chem.* **2010**, *31*, 455–461. [[CrossRef](#)] [[PubMed](#)]
107. Eberhardt, J.; Santos-Martins, D.; Tillack, A.F.; Forli, S. AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. *J. Chem. Inf. Model.* **2021**, *61*, 3891–3898. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.