red palm weevil

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Impact of Vitellogenin based dsRNA feeding on reproductive biology of red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae)

Abstract

Objective

Red palm weevil, *Rhynchophorus ferrugineus* is a crucial pest of date palm, in the Kingdom of Saudi Arabia including several other palm producing countries of the World. Recently, we have silenced the *vitellogenin* (*Vg*) gene by injecting the *RfVg*-based dsRNA and proved that RNAi technology can be used to manage RPW. The main challenge in RNAi-based plant protection methods is selecting a suitable tactic for successfully delivering the dsRNA.

Methods

As a result, in present study, RPW were fed *Vg*-based dsRNA and given drops in 9-10th instar larval stage to determine the effect of Vg-based dsRNA on RPW reproductive traits, such as pre-oviposition period, fecundity, oviposition period, post oviposition period, eggs size, and female and male life span.

Results

Results demonstrated that the *RfVg* gene function was successfully suppressed using Vg-based RNAi. when applied via drops, resulting in a significant decline in RPW eggs hatchability and *Vg* expression measured by quantitative real time PCR. However, when applied via diet, *Vg*-based dsRNA did not show any significant effect on fecundity, oviposition period, post oviposition period, eggs size, and female and male life span.

Conclusions

Based on present overall results and our previous findings, along with the documented information, we can conclude that *Vg*-based RNAi has a high potential for use as a target specific and eco-friendly technique for the sustainable control of RPW.

Keywords: RNAi, oviparous, date palm, *vitellin*, gene function, Saudi Arabia

Abbreviation

Rhynchophorus ferrugineus (*Rf*), *Vitellogenin* (*Vg*), RNA interference (RNAi), Vitellin (Vn), one-way analysis of variance (ANOVA), Red palm weevil (RPW),

1. Introduction

The red palm weevil RPW, *Rhynchophorus ferrugineus* (Olivier), (Coleoptera: Dryophthoridae) is a highly damaging pest of date palm in the Kingdom of Saudi Arabia including several other palm producing countries (Gomez and Ferry 1998). It has been estimated that in the Kingdom of Saudi Arabia, approximately 80,000 date palms are severly infested by RPW and posing a threat to other neighboring orchards (Al-Sheaby 2010) Infestations have been reported in more than half of the world's date palm growing countries, extending the entire Middle East (Faleiro 2006). On average, a female weevil lays about 48-139 eggs during its whole life period of 15-72 days (Aldawood et al., 2022).

In oviparous species, eggs production depends on the ability of *Vg* production and its accumulation in the oocytes. During reproductive phase, the female fat body produces a large amount the precursor Vg, which is subsequently reached to the oocytes through receptors (VgRs) through receptor-mediated endocytosis (Raikhel and Dhadialla 1992, Hagedorn et al., 1998, Sappington and Raikhel 1998, Snigirevskaya and Raikhel 2005, Tufail et al., 2005, Tufail and Takeda 2008, Tufail and Takeda 2009a, Tufail and Takeda 2009b, Tufail and Takeda 2012, Tufail et al., 2014, Tufail and Takeda 2018). The biosynthesis of *Vg* in most, if not all, insect species is controlled at the transcriptional level.

Taken together the *Vg* gene is the key component of egg production, and might be an appropriate target for developing more effective pest control methods for the crucial pest insects. For example, if the *Vg* gene function is knocked down by the RNA interference (RNAi) technology, the egg production can be stopped. RNAi is a natural process which silences specific genes before being translated. Efficient methods for gene silencing as a means of controlling pests have been successfully demonstrated in the laboratory (Price and Gatehouse 2008). RNAi technology, both in the form of crop spray or transgenic plants have the prospective to efficiently silence the targeted genes (Baum et al., 2007, Mao et al., 2007, Zhao et al., 2008) and must be especially important for the pests showing resistance to the pesticide or those having the hidden nature in the host plant.

Although, several kinds of measures have been tried to manage the RPW such as, pheromone traps, chemical control, injection of entomopathogenic nematodes (Shamseldean and Abd-Elgawad 1994), and fungus (Sutanto et al., 2022), however, none of them is able to completely eliminate/control this weevil. One of the major reasons this insect evades pest control measures is concealed reproduction of this insect within the palm tree protecting it from several interventions applied in the past. Under such situation, the alternative control strategies, especially with a molecular approach need to be sorted out. As mentioned above, RNAi technology has become tremendously a good tactic for studying the gene functions and to explore the potential genes for pest-control. We believe this target-oriented and environment-friendly approach would be the best choice for RPW control.

This manuscript is the part of the research project in which we isolated the *Vg* gene from the RPW to elucidate the reproduction mechanism of RPW and to exploit this molecular information to develop a system that can interfere the egg production using RNAi-technology as a target-oriented and environment-friendly pest control strategy for RPW in Kingdom of Saudi Arabia (see for detail our recently published manuscript in "Scientific Reports": (Rasool et al., 2021). Present study main objective was to testify the *Vg*-based dsRNA effectiveness through oral application against various biological parameters of RPW such as pre-oviposition period, fecundity, oviposition period, post oviposition period, eggs size, and female and male life span. We believe, RNAi-based strategy for control of RPW will be a great revolution in pest management system.

2. Materials and Methods

2.1 Red Palm Weevil Population

The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae), population (adult, larva, and pupa), primarily collected from the infested date palm orchards in the Aldierab region, of Saudi Arabia (24.4164°N, 46.5765°E). The infested date palm trees were inspected visually and signs of infestation were observed. After making sure that the inspected tree has RPW infestation; it was dissected and the RPW developmental stages were collected. These individuals were brought to the laboratory where they were reared on an artificial diet (Aldawood et al., 2022) to get the F_1 progeny. The RPW culture was established at a temperature of 25±1°C and 70±5% relative humidity throughout the study. The adult weevil from the F_1 progeny were allowed to copulate and lay eggs. To avoid the eggs from drying out, they were collected and kept in the petri dishes with wet filter paper at the bottom. These eggs were hatched into neonates which were provided the earlier described diet in order to grow and develop. These larvae were used for the *RfVg* based dsRNA feeding bioassay when they reached the 9^{th} and 10^{th} instars.

2.2 RfVg dsRNA feeding bioassy

In the present work the *RfVg*-based dsRNA feeding bioassay were performed on 9^{th} and 10^{th} instars old larval stage of RPW using the diet incorporation and direct drop feeding methods. The *RfVg* based dsRNA feeding bioassay was used in this study to assess their consequence on biological parameters and *Vg* expression in RPW. Briefly, the semi-synthetic diets, Diet-1 (artificial diet for RPW) and Diet-2 (diet used for *Spodoptera* spp. rearing), were cut into 2 cm^3 pellets and 4 µg (100 µl) of *RfVg* dsRNA was incorporated into each pellet and every pellet was kept in a separate cup. Before bioassay, RPW larvae were starved for 24 h and then fed on treated food pellets individually for 24 h. In another experiment, *RfVg* dsRNA 10 µl (4 µg) was delivered to RPW larvae by oral feeding using drop method. The *RfVg* dsRNA drops were delivered directly into the mouth of 24-h starved larvae using a micropipette. However, in the control nuclease free water was incorporated into artificial synthetic food and provided to the 24-h starved larvae. There were 10 replications and each replication consist of an individual

larva. Therefore, single larva was placed in each plastic cup and every plastic cup considered as a single replicate. After 24 h, the larvae were transferred to sugar cane in order to ensure cocoons (pupation), and followed daily till the RPW adults eclosion. Experiments were maintained in the incubator at 25°C ± 1 and 70 ± 5% relative humidity.

2.3 Biological studies to assess the impact of RfVg dsRNA

The newly emerged adults from the treated RPW larvae were paired and placed in a separate plastic container (1 kg) containing a bit of cotton overloaded with a 10% sucrose solution. Three pairs from each treatment were kept together, where they copulated and laid eggs until the RPW females survived. The pre-oviposition period, oviposition period, eggs laid/day, total number of eggs laid, egg hatching percentage, post-oviposition period, and life span were observed.

2.4 Validation through qRT-PCR

To verify the effect of *RfVg*-based RNAi on *Vg* gene expression, a (real-time reverse transcription-PCR) qRT-PCR analysis was performed by using the *RfVg*RTF and *RfVg*RTR primers previously used in our study (Rasool et al., 2021). Four experimental units, including (*RfVg* dsRNA-based diet-I, *RfVg* dsRNA-based diet-II, *RfVg* dsRNA drop, and control), were subjected to real-time PCR. There were three replicates for each treatment, each replicate having one RPW female, as well as three technical replicates. The reactions were prepared using SYBR® Green Supermix (BioRad) according to the standard protocol, and the real-time PCR was performed using the BioRad CFX-96 System.

2.5 Statistical analysis

To examine the differences between the four test groups (*RfVg* dsRNA-based diet-I, *RfVg* dsRNA-based diet-II, *RfVg* dsRNA drop, and control) for biological studies, one-way analysis of variance (ANOVA) was used followed by multiple-comparison testing with the least significant difference (LSD) test ($\alpha = 0.05$) by using SAS program ver. 9.2 (SAS 2008). Moreover, the qRT-PCR quantification results were analyzed and mean cycle threshold (CT) values were calculated to see the effect of dsRNA on the vitellogenin expression when fed to the RPW at larval stages.

3. Results

3.1 Effects of RfVg-dsRNA feeding on RPW biological parameters.

The *Vg* gene knockdown impact in adult RPW females as a result of feeding *RfVg*-dsRNA to 9th and 10th instars larvae in diet and drops was determined in terms of pre-oviposition, oviposition, and post-oviposition. The *RfVg*-dsRNA application significantly influenced some biological parameters in treated females. Results revealed that RPW larvae fed with *RfVg*-dsRNA by drops having significantly longer pre-oviposition period as compared to larvae that were provided with *RfVg*-dsRNA in diets and control (df = 3, F=6.59, P < 0.0041) (Fig. 1). There was no significant difference in the oviposition period between RPW larvae fed RfVg-dsRNA and controls (df = 3, F=0.15, P < 0.9291) (Fig. 2). Similarly, no significant difference (df = 3, F=0.60, P < 0.6268) was observed for post-oviposition period between RPW larvae feed on *RfVg*-dsRNA and control (Fig. 3).

3.2 Effects of RfVg-dsRNA feeding on No. of eggs laid, eggs hatching percentage, egg size, and adult female life span of RPW.

The effects of *Vg* gene knockdown in adult RPW females as a result of feeding *RfVg*-dsRNA to 9th and 10th instars RPW larvae in diet and drops was also assessed in terms of fecundity, eggs hatching percentage, and adult female life span. RPW larvae fed on *RfVg*-dsRNA showed no significant difference (df = 3, F=0.18, P < 0.9096) for no. of eggs laid between the control and *RfVg*-dsRNA treatments as drops and in diets (Fig. 4). But RPW larvae feed on *RfVg*-dsRNA irrespective of feeding methods revealed significantly lower eggs hatchability percentage (df = 3, F=3.22, P < 0.0509) as compared to control (Fig. 5). Results did not show any significant difference for adult female life span (df = 3, F=0.13, P < 0.9386) between RPW larvae feed on *RfVg*-dsRNA and control (Fig. 6). Moreover, results also not showed any significant difference for eggs length (df = 3, F=5021, P < 0.0031) as well as width (df = 3, F=1.56, P < 0.2081) between control and *RfVg*-dsRNA feeding through different methods (Fig. 7).

158 *3.3 Validation of RfVg gene expression*

159 The RNAi effect on *Vg* gene expression was validated through qRT-PCR. The dsRNA targeting
160 a unique region (locus 3538-3938) showing minor similarity with different insect Vgs fed in
161 diets and as drops to 9-10th instars RPW larvae. Real time PCR results indicated that the
162 expression level of *Vg* gene reduced significantly in all treated groups especially where dsRNA
163 was administered as drops in comparison with control group. The cycle threshold (CT) average
164 was recorded as 24, 19, 32, and 17 for *RfVg* dsRNA-based diet-I, *RfVg* dsRNA-based diet-II,
165 *RfVg* dsRNA drop, and control 15-days post-application, respectively (Fig. 8).

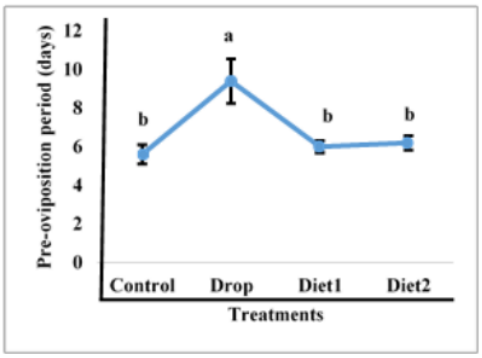

Fig. 1. Effects of *RfVg*-dsRNA feeding on pre-oviposition period.

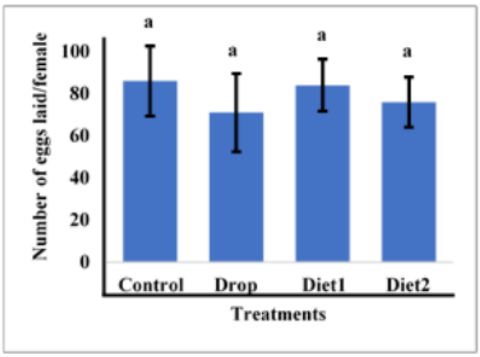

Fig. 4. Effects of *RfVg*-dsRNA feeding on mean number of eggs laid per female.

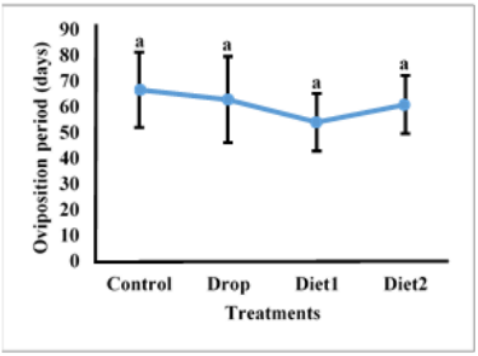

Fig. 2. Effects of *RfVg*-dsRNA feeding on oviposition periods.

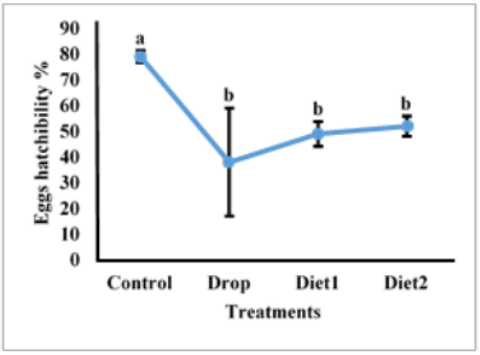

Fig. 5. Effects of *RfVg*-dsRNA feeding on eggs hatchability %.

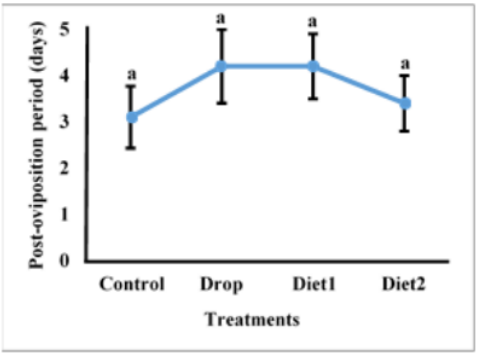

Fig. 3. Effects of *RfVg*-dsRNA feeding post-oviposition period.

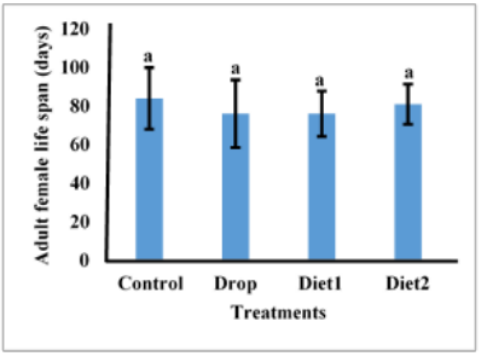

Fig. 6. Effects of *RfVg*-dsRNA feeding on adult female life span.

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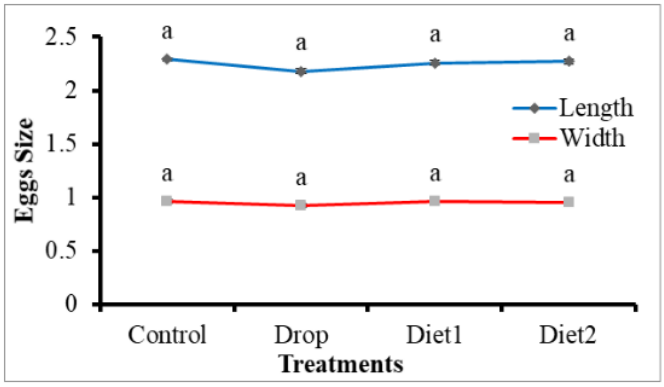
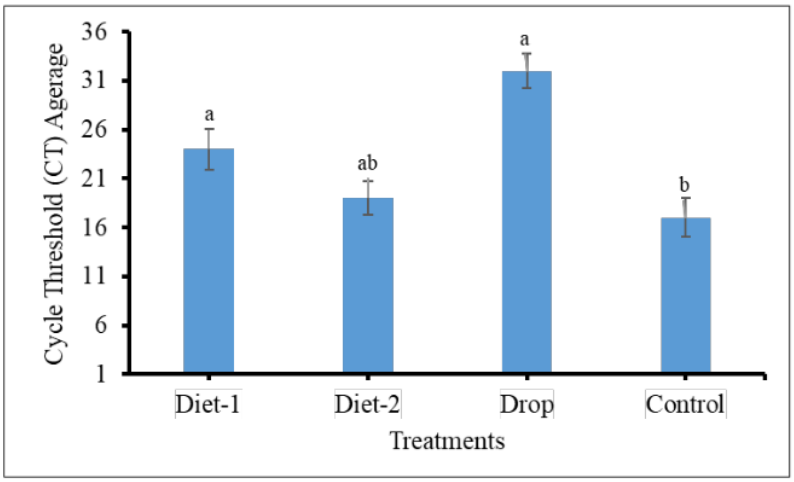


167 Fig. 7. Effects of *RfVg*-dsRNA feeding on egg size.



168 Fig. 8. The cycle threshold (CT) average recorded for different treatments after 15-days of
169 dsRNA application
170

4. Discussion

The red palm weevil is a major pest of palm trees all over the world. It causes tremendous economic losses to the palm farming community, especially in Middle East RPW proved a very deleterious pest of date palm trees. Despite numerous attempts to control RPW, no effective alternative method for eliminating this destructive pest has approved. Recent molecular studies revealed that RNAi can be used to silence any specific genes before being translated. RNAi has been successfully used as target specific and eco-friendly technique for controlling several insect pests. Gene silencing using RNAi for controlling insect pests has been successfully proven in the laboratory (Price and Gatehouse 2008). The use of dsRNA as a spray or in transgenic plants has a high potential for effectively silencing the targeted genes (Baum et al., 2007, Mao et al., 2007, Zhao et al., 2008) and work more efficiently against insect showing pesticide resistance. Recently, we have silenced *RfVg* gene by injecting the Vg-based dsRNA and demonstrated that RNAi could be used to manage RPW, the ruined pest of date palm trees (Rasool et al., 2021). Similarly, in the present study *RfVg*-based RNAi significantly impaired the reproductive performance in terms of pre-oviposition period, oviposition period, eggs laying, and eggs hatching in all treatments as compared to control.

In current study, pre-oviposition period was prolonged in females treated with dsRNA especially where dsRNA was offered through oral drops as compared to dsRNA provided in diets and control. Similar results were reported in our previous studies where dsRNA was delivered through dorsal inject (Rasool et al., 2021). Effects of *Vg* silencing on pre-oviposition period have also been reported in several insect pests (Coelho et al., 2016, Moriyama et al., 2016). Our previous findings along with those reported by others have shown that silencing of *Vg* gene expression resulted in the deformed eggs due to failure of Vg protein expression (Coelho et al., 2016, Moriyama et al., 2016, Rasool et al., 2021). The present study has also revealed similar effects on egg size of RPW.

In *RfVg*-dsRNA treated RPW females eggs hatchability% was zero as compared to control (Rasool et al., 2021). Though in present study eggs hatchability was not up to zero but reduced significantly in *RfVg*-dsRNA-treated groups. Some studies reported a significant decline in

fecundity of *RfVg*-dsRNA-treated female RPW (Tokar et al., 2014, Moriyama et al., 2016) but in present study we did not record any significant decrease in number of eggs laying in *RfVg*-dsRNA-treated groups.

We also tested the effect of *RfVg*-based RNAi on *Vg* gene expression, validated through qRT-PCR. The results of qRT-PCR indicated that the expression level of *Vg* gene reduced significantly in all treated groups especially where dsRNA was administered as drops in comparison with control group. In another study VgmRNA suppressed to 95.3% after 15-days of dsRNA application while suppression was further increased to 96.6% and 99.4% when tested after 20 and 25 days of dsRNA application through injection, respectively (Rasool et al., 2021). Successful suppression of *Vg* mRNA in RPW, in response to dsRNA application is a positive sign aimed at choosing VgRNAi for RPW management. Literature reveals that coleopteran insects are more vulnerable to RNAi as compared to other insect species such as *Diabrotica virgifera* (Baum et al., 2007), *Tribolium castaneum* (Whyard et al., 2009), *Leptinotarsa decemlineata* (Zhu et al., 2011), *and Anthonomus grandis* (Coelho et al., 2016). Based on the present promising results and literature reviewed we can suggest that *Vg*-based RNAi has a great potential to be used as a target specific and eco-friendly technique for the sustainable control of RPW and several other major insect pests. Future research should focus on developing a most suitable technique to deliver *RfVg*-dsRNA into palm/ date palm trees where it should be readily available to attack RPW.

5. Conclusions

The present study demonstrated that *Vg*-based RNAi effectively suppressed the function of *RfVg* gene, resulting into a significant decline in the hatchability % of RPW eggs and *Vg* expression as measured by qRT-PCR. Results did not show any significant effect of *Vg*-based dsRNA on fecundity, oviposition period, post oviposition period, eggs size, and female and male life span, when applied through diet. Our present results along with those of previous findings very clearly demonstrate that *Vg*-based RNAi has a great potential to be used as a target specific and eco-friendly technique for the sustainable control of RPW.

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Disclosure of any Conflict of interest

The authors declare that they don't have any type of cconflict of interest which might affect the present work.

References

Al-Sheaby, F., 2010. SABIC launches Red Palm Weevil workshop as part of its commitment to global corporate social responsibility. Online at: http://www. sabic. com/corporate/en/newsandmediarelations/news/20100331-2. aspx.

Aldawood, A.S., K.G. Rasool, S. Sukirno, et al., 2022. Semi-artificial diet developed for the successful rearing of red palm weevil: *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) in the laboratory. Journal of King Saud University-Science. 34 (7) 102272.

Baum, J.A., T. Bogaert, W. Clinton, et al., 2007. Control of coleopteran insect pests through RNA interference. Nature biotechnology. 25 (11) 1322-1326.

Coelho, R.R., J.D.A. de Souza Júnior, A.A. Firmino, et al., 2016. Vitellogenin knockdown strongly affects cotton boll weevil egg viability but not the number of eggs laid by females. Meta Gene. 9 173-180.

Faleiro, J., 2006. A review of the issues and management of the red palm weevil Rhynchophorus ferrugineus (Coleoptera: Rhynchophoridae) in coconut and date palm during the last one hundred years. International journal of tropical Insect Science. 26 (3) 135-154.

Gomez, V. and M. Ferry, 1998. Attempts at biological control of date palm pests recently found in Spain. Proceedings of the first regional symposium for applied biological control in mediterranean countries, Cairo.

Hagedorn, H.H., D.R. Maddison and Z. Tu, 1998. The evolution of vitellogenins, cyclorrhaphan yolk proteins and related molecules. Advances in insect physiology, Elsevier. 27: 335-384.

Mao, Y.-B., W.-J. Cai, J.-W. Wang, et al., 2007. Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. Nature biotechnology. 25 (11) 1307-1313.

Moriyama, M., T. Hosokawa, M. Tanahashi, et al., 2016. Suppression of bedbug's reproduction by RNA interference of vitellogenin. PloS one. 11 (4) e0153984.

Price, D.R. and J.A. Gatehouse, 2008. RNAi-mediated crop protection against insects. Trends in biotechnology. 26 (7) 393-400.

Raikhel, A.S. and T. Dhadialla, 1992. Accumulation of yolk proteins in insect oocytes. Annual review of entomology. 37 (1) 217-251.

Rasool, K.G., K. Mehmood, M. Tufail, et al., 2021. Silencing of vitellogenin gene contributes to the promise of controlling red palm weevil, *Rhynchophorus ferrugineus* (Olivier). Scientific reports. 11 (1) 1-12.

Sappington, T.W. and A.S. Raikhel, 1998. Molecular characteristics of insect vitellogenins and vitellogenin receptors. Insect biochemistry and molecular biology. 28 (5-6) 277-300.

SAS, 2008. SAS/STAT 9.2. user guide. SAS Institute, Carry, NC. USA.

Shamseldean, M.M. and M. Abd-Elgawad, 1994. Laboratory evaluation of six Egyptian isolates of heterorhabditid nematodes for control of the red palm weevil. Egyptian Journal of Applied Science. 9 (3) 670-679.

Snigirevskaya, E.S. and A.S. Raikhel, 2005. Receptor-mediated endocytosis of yolk proteins in insect oocytes. Reproductive Biology of Invertebrates, Vol. 12, Part B, CRC Press: 215-244.

Sutanto, K.D., M. Husain, K.G. Rasool, et al., 2022. Persistency of Indigenous and Exotic Entomopathogenic Fungi Isolates under Ultraviolet B (UV-B) Irradiation to Enhance Field Application Efficacy and Obtain Sustainable Control of the Red Palm Weevil. Insects. 13 (1) 103.

Tokar, D.R., K.A. Veleta, J. Canzano, et al., 2014. Vitellogenin RNAi halts ovarian growth and diverts reproductive proteins and lipids in young grasshoppers. American Zoologist. 54 (5) 931-941.

Tufail, M., Y. Nagaba, A.M. Elgendy, et al., 2014. Regulation of vitellogenin genes in insects. Entomological Science. 17 (3) 269-282.

Tufail, M., A.S. Raikhel and M. Takeda, 2005. Biosynthesis and processing of insect vitellogenins. Progress in vitellogenesis. Reproductive biology of invertebrates. 12 (part B) 1-32.

Tufail, M. and M. Takeda, 2008. Molecular characteristics of insect vitellogenins. Journal of insect physiology. 54 (12) 1447-1458.

Tufail, M. and M. Takeda, 2009a. Insect vitellogenin/lipophorin receptors: molecular structures, role in oogenesis, and regulatory mechanisms. Journal of insect physiology. 55 (2) 88-104.

Tufail, M. and M. Takeda, 2009b. Insect vitellogenin/lipophorin receptors: molecular structures, role in oogenesis, and regulatory mechanisms. Journal of insect physiology. 55 (2) 88-104.

Tufail, M. and M. Takeda, 2012. Hemolymph lipoproteins: Role in insect reproduction. Hemolymph proteins and functional peptides: Recent advances in insects and other arthropods. 1 3-19.

Tufail, M. and M. Takeda, 2018. Vitellogenesis and yolk proteins in insects.

Whyard, S., A.D. Singh and S. Wong, 2009. Ingested double-stranded RNAs can act as species-specific insecticides. Insect biochemistry and molecular biology. 39 (11) 824-832.

Zhao, Y., G. Yang, G. Wang-Pruski, et al., 2008. Phyllotreta striolata (Coleoptera: Chrysomelidae): Arginine kinase cloning and RNAi-based pest control. European Journal of Entomology. 105 (5) 815.

Zhu, F., J. Xu, R. Palli, et al., 2011. Ingested RNA interference for managing the populations of the Colorado potato beetle, Leptinotarsa decemlineata. Pest management science. 67 (2) 175-182.

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