Research Article

Morphometric Life Attributions of Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) under two Controlled Consecutive Temperatures

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Abstract | The present research was carried out at (20±2°C) and (30±2°C) temperatures under laboratory conditions during, 2023 to determine the morphological attributions of *Rhynchophorus ferrugineus*. The female eggs color patterns were milky white oval, and cylindrical. The length and width of eggs (2.56±0.40) (1.18±0.28) were noted at (30±2°C) followed by (2.33±0.42), (1.102±0.22) mm, at (20±2°C). The newly hatched larvae were white-brown and gradually turned into dirty-brown at later stages. The 1st instar stage measurement was recorded (0.98±0.31), 2nd (172±0.40), 3rd (2.86±0.52), 4th (0.63±0.25), and 5th (3.54±0.70) mm at (30±2°C). Similarly, at (20±2°C), the 1st instar stage measured (0.45±0.21), 2nd (0.67±0.28), 3rd (1.20±0.37), 4th (2.10±0.45), and 5th (2.96±0.64) mm in size. The larvae weight of each stage was recorded (0.18±0.24), (0.99±0.23), (1.42±0.25), (2.96±0.43) and (4.44±0.74) in grams at (30±2°C). However, at (20±2°C), larvae weight was calculated (0.15±0.09), (0.78±0.18), (1.12±0.27), (2.165±0.35) and (3.98±0.66). The radish-brown pupae body length was recorded (3.65±0.45), its weights reported (4.94±0.72), and (4.66±0.80) in grams. The radish-brown black colored patches of male length were (2.98±0.51), female (3.22±0.60) at (30±2°C) as followed (3.14±0.31), (2.87±0.29), (3.10±0.58) mm at (20±2°C). Five days old adult males and females weights were (2.16±0.31), (2.52±0.40) and (1.87±0.29), (2.10±0.48) at both temperatures, in three replications. This species of insect pest is sporadic in its feeding habits and habitat, causing great losses to the economic heritage of many nations. It is further suggested that more work should be carried out on different survival patterns of this invasive pest species of date palm orchards.

Received | July 23, 2024; **Accepted** | August 26, 2024; **Published** | August 30, 2024

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Citation | Mangrio, W.M., Y. Buriro, H.A. Sahito, F.A. Jatoi and F.I. Sahito. 2024. Morphometric life attributions of red palm weevil, *rhynchophorus ferrugineus* (olivier) under two controlled consecutive temperatures. *Pakistan Journal of Agricultural Research,* 37(3): 267-274.

DOI | https://dx.doi.org/10.17582/journal.pjar/2024/37.3.267.274

Keywords | *Controlled Temperatures, Dirty brown larvae, Milky-white colored eggs, Red palm weevil, Sporadic pest, White-brown larvae*

Introduction

The date palm (*Phoenix dactylifera* L.), originated 5,000 years ago in Mesopotamia, is a symbol of culture, and one of the oldest perennial fruit trees, broadly cultivated in different regions of the world (Fernandez-Lopez *et al.*, 2022). This oldest fruit crop has been cultivated in South Asia, North Africa, Central America, and the Middle East, strongly characterized by well-developed root sys-

tems in drained and sandy soil (Kamel *et al*., 2022). Date palm crop grow well in marginal environments, their fruit and seeds are a rich source of income for many countries (Tanghberg, 2012). In the case of established industrialization, Pakistan holds the 5th position and it is a core region for date production with a pivotal role in the economy of the country (Jatoi *et al*., 2021), but Khairpur of Sindh is regarded as the homeland, biodiversity center and main hub, more than three hundred novel varieties of date palms are being grown here (Mangrio *et al*., 2024).

The physical properties and quality of date fruit usually can be determined by color, size, texture, shape, fruit, and seeds of date palm are rich sources of carbohydrates and vitamins (Sahito *et al*., 2017), also several other micro and macro nutrients (Mallah *et al*., 2017). The date fruit has many positive effects on human physiology (Kousar *et al*., 2022). Many insect pest species adversely affect date palms and their fruit, causing negative effects on quality and quantity, among these the most destructive is RPW, causing wide economic hurt (Kubar *et al*., 2017).

Rhynchophorus ferrugineus, additionally referred to as the Asian palm weevil, belongs to the family Curculionidae, order, Coleoptera, an invasive destructive pest of palm trees in various ecosystems and a global threat to palm plantations in subtropical and tropical zones (Najim *et al*., 2024). This devastating pest species emerged early in the twentieth century from Southeast Asia, and has spread to North Africa, Middle East, America, and Europe, but now exhibits global distribution, causing extensive heavy losses (Aziz, 2024). Red palm weevil, the world's most persistent pest of palms for a few decades, rapidly expanded its geographical distribution, and host plant range has been reported from twenty eight Asian, fourteen European, six African, five Oceania, two Central and one North America (Aziz *et al*., 2022).

Usually, the larvae stages of the insect pest species are voracious feeders to their hosts, feeding entire content causes deformities, and weakness, ultimately leading to fall and demise (Mangrio *et al*., 2020). It is a difficult and enormous challenge to detect and manage infested palm trees in palm plantations due to plant height, inside feeding, host species diversity, strong survival capability in altered environments, locomotive tendency, long life cycle with multiple generations, and larvae boring activity on host plants (Kharim *et al*., 2022). Red palm weevil acts as a major pest causing, immense economic and biodiversity losses. Now different companies, governments, researchers across multiple countries are taking a keen interest in developing control strategies to restrict further damage from this potential pest species (Manee *et al*., 2023). This study dealt with morphological description with the hope that this scientific information may aid in a better diagnostic understanding of the red palm weevil.

Materials and Methods

Collection of insect pest data

In the present research work, the pupae populations of RPW were collected from date palm infested orchards at Prof. Dr. Abdul Khalique Jatoi farm, Taluka Kingri, during 2023. The pest populations were captured by using a handpicking method with the help of date palm workers. The discussed area is regarded as a robust date palm zone in the district Khairpur Mirs, located at 27.6507°N, and 68.5724°E.

Population culture of Rhynchophorus ferrugineus

For morphological study of the red palm weevil, collected pest populations were brought to the diagnostic Laboratory of Entomology, Department of Zoology, SALU, Khairpur Mirs. The pupae population was placed individually in buckets under two variable temperatures for culture maintenance. To facilitate the newly emerged adult males and females, inside the bucket soft date tissue particles were kept.

Culture rearing and diet of the RPW

Two rooms were settled at (20±2°C) and (30±2°C) temperatures and (70 to 80%) relative humidity. A total of (n=10) two days of male and female insect pests from each sex were released into large sized (3×33ft) wooden cages for oviposition. The dry and semi-dry dates, sugarcane stems, and internal soft bark of date palms were given to facilitate suitable breeding inside the cage. For diet purposes, all adults were given 10% honey solution on cotton pouches.

Collection of fresh eggs of RPW

The fresh females laid eggs were collected with the help of camel hair brush and kept on wet tissue papers to overcome the chances of dryness. Then the eggs of RPW were assembled and subsequently kept separately into six petri dishes (15x15cm) in diameter. For egg hatching and other laboratory examinations, two

laboratory rooms were arranged for each treatment at (20±2˚C) and (30±2˚C) temperatures. An equal number of eggs was kept in both temperature conditions in three equal petri dishes individually.

Newly hatched Larvae, Pupae and Adults

The freshly hatched larvae were carefully transferred through a camel hairbrush and shifted into net and cleaned plastic petri dishes by putting similar food in both temperature conditions. For morphological description of each specific stage of life, the data of the RPW were repeated three times, and fresh food was given when they consumed or devoured previously. Later on, when larvae convert into the stage of pre-pupae, then for cocoon formation, small pieces of sugar cane stems were offered. Later on, cocoons containing pupae were carefully placed into net and clean sterilized glass petri dishes for adult emergence, and observed on a daily basis. Through a handpicking method, new emerging adults were captured and kept in jars individually in each replication.

Morphological studies of RPW

For the determination of all morphological characteristics from egg to adult of the red palm weevil under two controlled temperatures, the egg period was assessed from egg lying up to egg hatching and attention was given to the color and shape of eggs. On starch paper sheet covered with cellulose, (n=10) pairs of newly emerged adults of RPW were kept individually from egg to adult examination. The period from the egg lying, up to the egg ceasing and daily laid eggs were measured and counted in the early morning (9:30 to 11:00 a.m.). All stage larvae were kept in (110 mm) petri dishes, and attention was given to the time duration and conversation from one to the next stage with diagnostic characteristics up to pupae stages. The egg, larvae, pupae, and adults of the RPW were measured in mm. When larvae become sluggish and stop feeding, they convert themselves into pre-pupae, pupae and post-pupae stages. All life associated stages were measured and morphometric were examined. From post-pupae, adults emerged, and it's all associated color patterns were noted. For the determination of sexual dimorphism (n=30), pupae cocoons were kept to emerge out either male or female. The life cycle from egg emergence up to death of the RPW, the longevity of adult males and females were recorded. From egg to adult, ANOVA of the insect pest was subjected to analysis by version SXW 8.1, USA software. The morphometric pictures of the different stages were captured through a source CPU monitor connected to a digital camera. The life cycle stages and body diagnostic characteristics with length and width measurement differences were calculated at ($p< 0.05$). The figures were made by the source of vivo Y21 Android Smartphone and Origin 2017, 64 Bit software.

Results and Discussions

Morphological characteristics of RPW

The results of morphological characteristics of the red palm weevil under two controlled temperature conditions were carried out at (20±2˚C) and (30±2˚C) under laboratory conditions. The main outcomes of the current research are:

Egg color patterns of RPW

In this research study, the evaluation of all life stages of the *Rhynchophorus ferrugineus* in morphological differentiations, color patterns, and measurements were taken for better determination. The fresh females lay eggs from the ovipositor, which is situated at the abdomen end. The ovipositor is an abdominal body part, suited to insert eggs inside the trunks, wounds, fissures, and crevices of date palm trees. The eggs were smooth, oblong, shiny, milky-white, oval, and sparkling as shown in (Figure 1).

Figure 1: *a; Egg of red palm weevil.*

Eggs range in size of RPW

The female adult's maximum mean length of eggs were recorded as (2.56±0.40) mm at (30±2˚C) and (2.33±0.42) mm at (20±2˚C), respectively. Same way, the mean width of the eggs were calculated (1.18±0.28) mm at (30±2˚C) and (1.102±0.22) mm at (20±2˚C), respectively. When egg length and width

mean data was subjected to analysis calculated with significant differences in size (DF= 1; F= 16.17; P= 0.01) at both controlled laboratory temperatures as shown in (Figure 2).

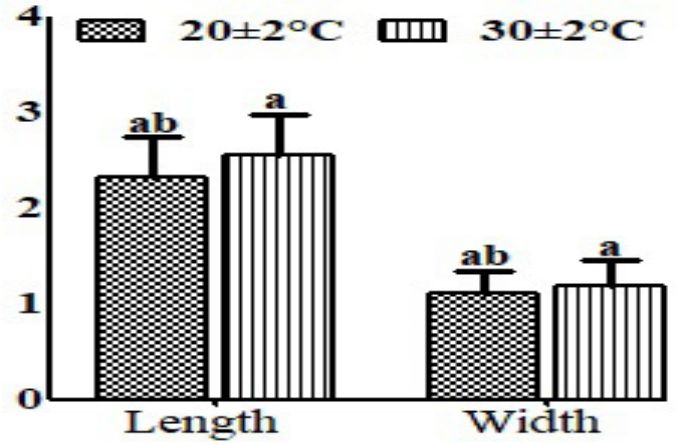

Figure 2: *Variation in egg size at two distinct temperatures.*

Larvae color patterns from 1st to 5th stages

The new hatchlings can grow long, and can be perceived by their earth colored head and slightly pale yellow-white body, legless, and have a chitinous head more obscure brown than the rest body. All stage larvae feed on palm tissues from tunnels while feeding filled become frass, eventually, find their way into the palm heart. The larvae's body is made from different secretions and the 1st instar stage was found slightly white with brown patches but gradually turned dirty-brown at later stages. The larvae mouthparts were found in round shaped emphatically chitinous with strong cone-shaped jaws used to tunnel from the axils of the passes onto the crown, where they feed ravenously, as depicted in (Figure 3).

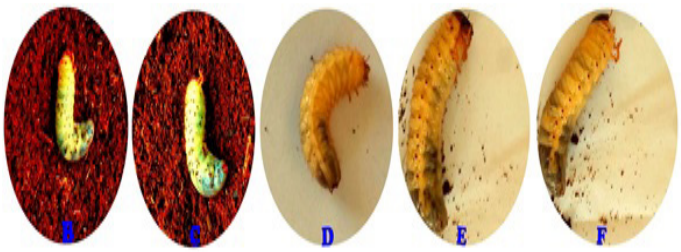

Figure 3: *b; 1st stage, c; 2nd stage, d; 3rd stage, e; 4th stage, f; 5th stage of red palm weevil.*

Larvae measurement of RPW

All stages of newly hatched larvae at both temperatures were measured in millimeters. The length of the 1st stage larvae recorded (0.63±0.25), 2nd stage (0.98±0.31), 3rd stage (1.72±0.40), 4th stage (2.86±0.52), 5th stage (3.54±0.70) at (30±2°C), respectively. Whereas; at (20±2°C) the 1st stage larvae were measured at (0.45±0.21), 2nd stage (0.67±0.28), 3rd stage (1.20±0.37), 4th stage (2.10±0.45), 5th stage (2.96±0.64) mm, respectively. ANOVA of all pairwise larvae comparisons with significant differences between the all stage larvae treatments and divided into homogenous groups at (P< 0.05), at two distinctive temperatures, as depicted in (Figure 4).

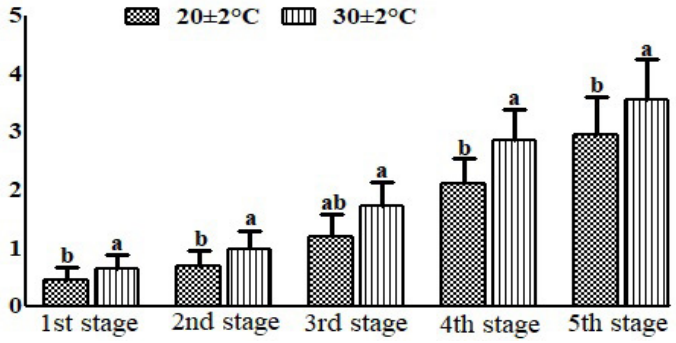

Figure 4: *Different stage larvae length variation at two different controlled temperatures.*

Appearance of RPW cocoon, pupae, and adults color patterns

The last stage of RPW larvae converts into pupae stages and construct an oval-shaped nest with fibers of cocoon. The larvae develop into cocoons which can be found commonly on trunk of the palm trees and hide at the base of palm fronds. After completion of the pupae period, adults emerge from the cocoon of fibers, which is ruddy brown portrayed by a bended platform with a nose like appearance. Dim colored spots were found on the upper side of the body at the central region. The head platform was rounded in length. The male's dorsal apical nose portion is covered with tuft hairs, the rostrum is stout, smaller than shiny black spotted pronotum, wider elytra, and black pygidium. The female's nose is uncovered, slimmer, bent, and longer than the males with radish-brown black margin colored body, as shown in (Figure 5).

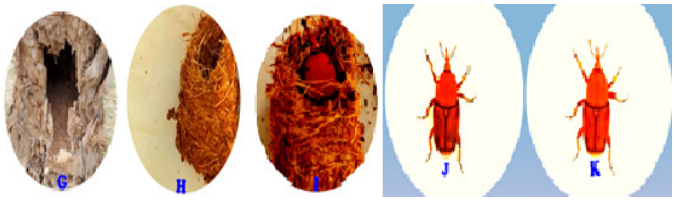

Figure 5: *g; larvae infestation, h; cocoon possess pupae, i; adult emerging, j and k; adult male/female.*

Pupae and adult measurement of RPW

The pupae and adult stages of the RPW were measured in mm. The mean length of pupae was recorded

(3.65±0.45) at (30±2°C) and (3.14±0.31) at (20±2°C), respectively. However, in the males, the maximum mean length was (2.98±0.51) recorded at (30±2°C) and (2.87±0.29) at (20±2°C), respectively. In the case of females, the maximum length recorded (3.22±0.60) at (30±2°C) followed by (3.10±0.58) at (20±2°C), respectively. The ANOVA of pupae, adult male, and female were statistically analyzed found significant difference was recorded in their body length ($P< 0.05$) in all replications, as justified in (Figure 6).

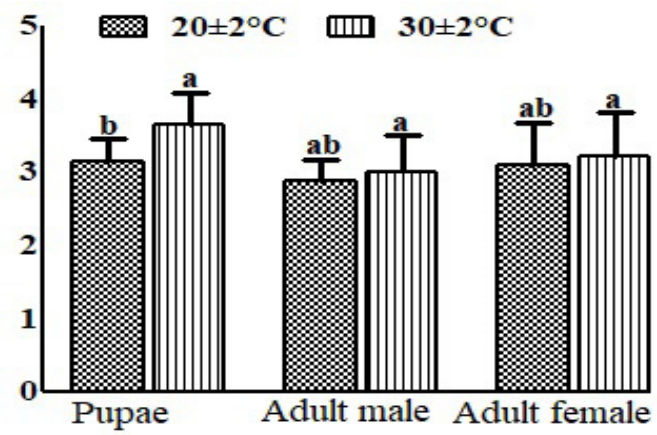

Figure 6: *Pupae, adult male and female length variation at two different controlled temperatures.*

Larvae and pupae weight measurement in grams of RPW

The weight of newly different larvae stages and pupae were measured in grams. The maximum weight of 1st stage larvae was calculated (0.18±0.24), 2nd stage (0.99±0.23),3rd stage (1.42±0.25),4th stage (2.96±0.43), 5th stage (4.44±0.74) in grams at (30±2°C). Similarly, at (20±2°C), the maximum weight of 1st stage larvae was recorded (0.15±0.09), 2nd stage (0.78±0.18), 3rd stage (1.12±0.27), 4th stage (2.165±0.35), 5th stage (3.98±0.66), in grams, respectively. While as, the maximum pupae weight was (4.94±0.72) recorded at (30±2°C) followed by (4.66±0.80) at (20±2°C) in grams. ANOVA of the larvae and pupae weight in grams were statistically analyzed at ($P< 0.05$). LSD test of all pairwise larvae and pupae weight comparisons found differences in both treatments, as shown in (Figure 7).

Adult male and female weight measurements of RPW

The weight measurements of the five days old adult male and female RPW were taken in grams. The maximum weight of males (2.16±0.31) was noted in grams at (30±2°C) followed by (1.87±0.29) grams at (20±2°C). Same way, the maximum weight in grams of females (2.52±0.40) was recorded at (30±2°C) as compared (2.10±0.48) grams at (20±2°C), respectively. The data showed tremendously significant difference in grams between males and females in all replications under both laboratory controlled treatments, as depicted in (Figure 8).

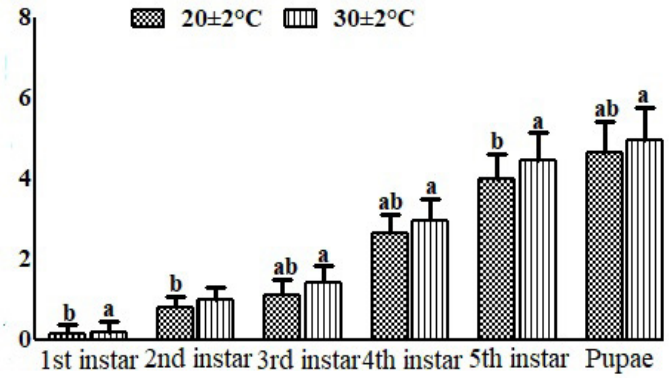

Figure 7: *Larvae and pupae weight in grams at two distinctive temperatures.*

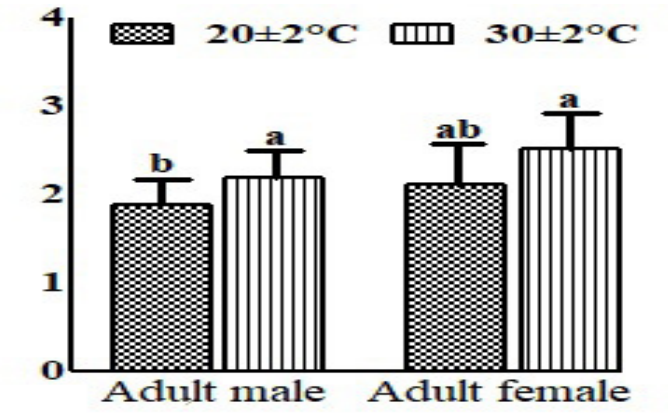

Figure 8: *Adult male and female weight in grams at two distinct temperatures.*

The date fruit is considered as a complete food with high nutritional value, in case of Pakistan, this valuable crop is mainly cultivated in Khairpur, Sindh. However, in the present scenario, its production is going to decrease, due to severe attacks by biotic and abiotic factors, of which insect pests are key declining factors, they detect violate compounds, released by host plants and reach to deposit eggs, as a resultant cause great losses every year (Sahito *et al.*, 2017). Addressing the importance of dates and keeping the view of damaging elements such as RPW, this research was designed and performed to observe the morphological traits of this pest species. Further, morphometric characteristics revealed that the eggs of RPW reported oval, cylindrical shaped, and milky white correlates. (Farooq *et al.*, 2019), documented

that the RPW, a significant pest of palm plantations globally, hatch oval-shaped eggs in the crown of date palms, inside divots, and soft tissue fissures, and their larvae progressively create deep tunnels by chewing delicate tissues.

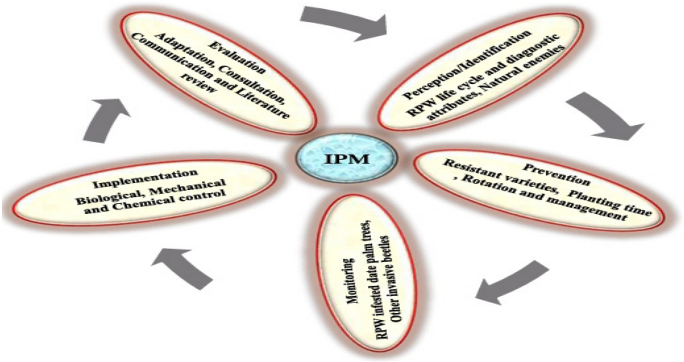

Figure 9: *Integrated Pest Management strategies.*

Newly hatched larvae were found whitish with brown patches at 1st and 2nd stages but from 3rd up to 5th stages gradually turned yellow-brown legless body with an earth-colored or brownish head more obscure than the rest body with chitinous mouthparts (Kubar *et al*., 2017), documented RPW yellow-white eggs 2.5 mm in length, 1st, 2nd, 3rd stage larvae in yellow-white color with light-brown head, measured 2.5 mm, 10.5 mm, 14.6 mm, but 4th and 5th stage in pale-yellow colored body with dark-brown head measured 20.9 mm, 30.15 mm from the same area. At the last stage larvae form ruddy-brown portrayed blended fibrous cocoon, where larvae convert into pupae finally an adult. (Jatoi *et al*., 2020), reported light-yellow oval shaped egg and larvae of lesser date moth found 1st stage larvae more elongated than the rest, much darker pre-pupae with black pigmentation, pupae with puffy hairs, and adult males comprised dirty colored body. The weight of larvae, pupae, and adults was recorded at both temperatures. (Kousar *et al*., 2021), documented whitish egg color of saw-toothed grain beetle but gradually becoming dark-brown, 1st stage larvae are whitish but 2nd and 3rd stage whitish colored with brown head, and 4th stage contains brownish body with darker head, dark-brown pupae covering cocoon, and adult comprise slender shaped dark-brown body. The maximum growth in length, width, and weight of all diagnostic characteristics was recorded at (30±2˚C) compared to (20±2˚C). This is compared with the previous reports of (Mastoi *et al*., 2023), that temperature fluctuations gradually affect the life expectancy of the pest species. Including life stage diagnostic characteristic study, it is essential to adopt IPM advanced strategies (Figure 9) for this invasive species because there is limited information regarding the morphometric and other life attributes of RPW.

Conclusions and Recommendations

This research work was conducted to determine the life diagnostic characteristics such as; eggs, larvae, cocoon, pupae, adult males and females of the RPW under (30±2˚C) and (20±2˚C). In both ranges of temperature, the length, width, and weight were measured and reported with significant differences. It was concluded that at (30±2˚C), pest species morphologically grow and develop sufficiently but below and above the temperature range creating diagnostic diversity. However, it is strongly needed to control this insect pest at their native temperatures using sustainable cost-effective, eco-friendly advanced IMP strategies.

Acknowledgements

The authors would like to express their most profound gratitude and sincere thanks to the date palm farmers of Prof. Dr. Abdul Khalique Jatoi farm for pest collection by handpicking method and jolly support. The authors would like to thank, Department of Zoology, for providing access to the research environment in Entomology Laboratory.

Novelty Statement

Pakistan is an agricultural country, known as the robust zone for date palm productivity with massive economic worth. But this valuable crop is gradually harmed by a variety of destructive pest species of which the red palm weevil is invasive. It is direly needed to study the life cycle and morphometric characteristics and should be introduced advanced eco-friendly IMP strategies.

Authors Contribution

Wali Muhammad Mangrio: Principal author of this scientific documentary, who played a significant role in pest collection, laboratory examination, and manuscript write-up.
Yasmeen Buriro: Participated in data collection and observed all stages of the life cycle of pests at both temperatures.

Hakim Ali Sahito: Visualization, analysis, and supervises the biological and morphological study.
Faheem Ahmed Jatoi: Performed conceptualization, validation, software, data curation, and methodology.
Fahmeeda Imdad Sahito: Designated, conceived, and arranged tools, helped in proofreading and final editing.

Data Availability Statement

The data associated with this research paper can be directed to the corresponding author.

Publication Consent

All responsibilities for publishing this research paper are accepted by the corresponding author.

Conflict of Interest

The corresponding author declares that they do not have any conflict of interest or subject matter in this scientific study.

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