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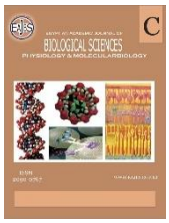

Biochemical Effects of Synthetic Pure Phenolic Compounds Glycyrrhetinic Acid, Rutin, and Quercetin on *Locusta migratoria migratorioides* (Orthoptera: Acrididae)

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ABSTRACT

The African locust *Locusta migratoria migratorioides* is a highly destructive agricultural pest. To develop environmentally safe and low-toxic pesticides, this study investigated the effects of glycyrrhetinic acid, rutin, and quercetin on physiological balance of *L. migratoria*. The study examined their impact on antioxidant/detoxifying enzymes, nutrition, and reproduction. The research evaluated growth, development, and biochemical aspects from the second instar to adulthood. Levels of reduced glutathione (GSH) and the catalytic activities of glutathione peroxidase (GPx), glutathione reductase (GR), glutathione transferase (GST), and catalase (CAT) were measured. The findings showed significant body weight reduction in adult *L. migratoria* across all compound concentrations. Increased rutin concentration led to decreased feeding indicators. GSH levels and antioxidant enzyme activities, particularly GST and CAT, increased significantly during the adult stage. Reproductive indices were notably affected, with rutin and quercetin causing decreased fecundity, fertility, and sterility rate (CS %). Despite the observed effects on *L. migratoria*'s physiology and reproductive capacity, the study highlighted the species' robust protective defence system and high adaptability. The examined compounds interfered with insect development and reproduction, suggesting their potential for pest management.

INTRODUCTION

Locusts are widely recognized as the most destructive agricultural pests globally. Among the locust species, *Locusta migratoria* L. (Orthoptera: Acrididae) has been prevalent since ancient times and holds the status of being the most widely distributed. Geographically, the migratory locust subspecies can be categorized into two species: the Asian *Locusta migratoria migratoria* (Linnaeus, 1758) and the African, *Locusta migratoria migratorioides* (Reiche and Fairmaire, 1849) (Le Gall *et al.,* 2019). The African locust, known for its consumption of both crops and weeds, is particularly notorious as one of the most economically damaging pests. It poses a significant threat to agriculture, especially in regions such as Egypt's Western Desert, the Assiut Governorate in Upper Egypt, and new land reclamation projects like Sharq Al-Owainat and Toshka (Soliman *et al.,* 2019; El-Samad *et al.,* 2022).

Consequently, the potential use of *L. migratoria* in environmental toxicity studies has gained considerable attention due to its innate advantages, such as short life cycles and adaptable living requirements (Arafat *et al.,* 2023).

For pest control, insecticides from major classes like organophosphates and carbamates have traditionally been employed. However, their detrimental effects on the environment and human health have prompted search for alternative agents that are environmentally friendly, rapidly biodegradable, and exhibit low toxicity towards the target insect pests under investigation (Said *et al.,* 2018; Huang *et al.,* 2020; Du *et al.,* 2022; Tanaka, 2022).

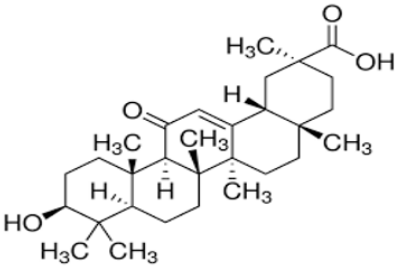
Plants have evolved various defence mechanisms to counter insect and pathogen attacks. One such defence mechanism involves the production of secondary metabolites, which play a protective role in plant resistance against a wide range of insects. Secondary metabolites, including phenolics, terpenoids, and alkaloids, have profound effects on insect behaviors. They can inhibit insect feeding, act as repellents, and exhibit toxicity towards insects. Additionally, these metabolites can interfere with insect growth and development (Wu *et al.,* 2015; Huang *et al.,* 2020; Yuan *et al.,* 2020; Gao *et al.,* 2022). The continuous consumption of plant phenols by insects raises an intriguing question: Do insects possess mechanisms to tolerate and detoxify them through specialized enzymes?

Insects, particularly herbivorous species, are susceptible to various stress factors, both abiotic and biotic, which can significantly impact their development and growth (Khan *et al.,* 2022). Their feeding habits make them more vulnerable to oxidative damage, compounded by the presence of reactive oxygen species (ROS) generated through aerobic metabolism and other sources (Dampc *et al.,* 2020). Diet stress arises from exposure to pro-oxidant chemicals such as phenolic compounds, which can induce physiological changes and alter gene expression in herbivorous insects. The success of phytophagous insects relies on their ability to adapt to diverse biotic stresses caused by different types of phenolic substances and modify their defence mechanisms accordingly (Gao *et al.,* 2022).

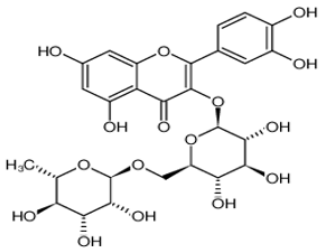
Insects, like all living organisms, possess an effective antioxidant defence system that maintains a regulated balance between ROS production and oxidative damage to ensure cellular homeostasis. This balance is crucial for insect development and other life activities. Non-enzymatic antioxidants, such as reduced glutathione (GSH), and antioxidant enzymes, including glutathione transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR), and catalase (CAT); play essential roles in this defence system. Disruption in the antioxidant system can impair its function, ultimately compromising the survival of the pest. Therefore, studying the activity of these enzymes can provide valuable insights for insect control strategies (Sahoo *et al.,* 2016; Hamed *et al.,* 2019; Silva *et al.,* 2021; Fahmy *et al.,* 2022).

Preliminary studies were conducted on the migratory locust, *L. migratoria*, by feeding the insect with alfalfa leaves moistened with an aqueous extract of the roots of *Glycyrrhiza glabra* (liquorice roots). The insects completely refrained from feeding, even upon dilution of the extract concentration. Understanding the key compounds present in *G. glabra* root extract and studying their effects on migratory locusts can provide insights into why insects exhibit feeding inhibition when treated with *G. glabra* root extract, ultimately weakening and eliminating the insect. Previous research by Hamed *et al.* (2019) conducted high-performance liquid chromatography (HPLC) analysis on aqueous extracts of *G. glabra* roots, revealing that the main components in the extract are glycyrrhetinic acid (1.43 mg/g dry plant), rutin (0.55 mg/g dry plant), and quercetin (0.14 mg/g dry plant), accounting for 61.6%, 23.7%, and 6% of the total phenolic content, respectively.

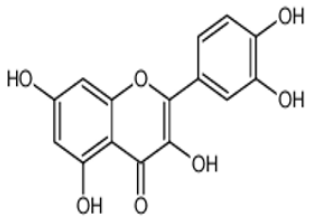
The aim of this study was to investigate the effects of glycyrrhetinic acid, rutin, and quercetin on antioxidant activity, detoxification enzyme activity, and oxidative stress equilibrium. By analyzing these mechanisms, which bear similarities to the mode of action of many insecticides used in insect control, this study aimed to understand the potential of these compounds in regulating insect populations. The chemical structures of the glycyrrhetinic acid, rutin, and quercetin are shown in Figure 1. Additionally, we examined the impact of these compounds on essential nutritional and reproductive parameters during *L. migratoria* development, from the early stages to the adult female stage. This research contributes to the development of new strategies for insect control and paves the way for future advancements in this field.



Glycyrrhetinic acid



Rutin



Quercetin

Fig. 1: Chemical structures of glycyrrhetinic acid, rutin, and quercetin. The chemical structures were drawn using ACD/ChemSketch 2018.2.1 program (ACD/Labs, Toronto, ON, Canada).

MATERIALS AND METHODS

Chemicals:

Bovine serum albumin fraction IV (BSA) (≥98%), reduced glutathione (GSH) (≥95%), oxidized glutathione (GSSG) (≥98%), and 1-chloro-2, 4-dinitrobenzene (CDNB) were purchased from Merck Company (Germany). Nicotinamide adenine dinucleotide phosphate reduced form (NADPH) and 18 α-glycyrrhetinic acid (≥95%), rutin (≥94%), and quercetin (≥95%) were obtained from Sigma-Aldrich Company (USA). All other chemicals used in this study were of the highest commercially available purity.

The African migratory locust species *L. Migratoria migratorioides* (Reiche & Fairmaire 1849) (Orthoptera: Acrididae) was identified and obtained from Locust Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki. Giza. Second instars used in this study were collected during the summer season (June to October) 2022. Insects were reared in the laboratory in wooden cages covered with thin ventilation nets under standard temperature conditions: 30 ± 5°C; humidity: 50-65%; photoperiod: Light: dark 12:12 h with unlimited access to water, food, and daily cage cleaning (Hill and Taylor, 1933).

Insects feed on the normal diet of alfalfa leaves, *Medicago sativa* (Vapalis: Fabaceae) or corn, *Zea mays* (Poales: Poaceae) depending on the season (Muhammad *et al.*, 2022). The experiment was designed with a total of 54 second instar

nymphs. They were divided into 6 treatment groups, with 9 insects assigned to each group. Each treatment group had 3 replicates, and each replicate consisted of 3 insects. Additionally, there was a control group of 54 nymphs that were not subjected to any treatment and were fed naturally. The growth of insects was monitored from the second instar to the adult stage. At each stage (second instar, fifth instar, and adult), both the treated insects and the control group were collected. The collected insects were weighed and then stored at -20 °C for further analysis. Females only were selected for the experiments.

Two concentrations of glycyrrhetinic acid (0.1mM, 1 mM), rutin (5mM, 10 mM), and quercetin (2 mM, 30 mM) were used for feeding of the second instar of *L. migratoria*. About 75g of fresh corn leaves (*Zea mays*) were dipped in 100 ml of each concentration for 5 minutes; air dried at room temperature, and then introduced to the aerated plastic vessel (8 × 10 × 10 cm) contained 3 locusts each. Treated corn leaves were fed to the insects for 24 h. Control insects were fed on corn leaves dipped in distilled water only. Insect behaviour modification activity and feeding ability were monitored 48 h after treatment with glycyrrhetinic acid, quercetin, and rutin.

Determination of Body Weight Changes in *L. migratoria* During Different Developmental Stages:

The effects of glycyrrhetinic acid, rutin, and quercetin on growth, development, and survival of treated insects from second instar to the adult stage were studied. After feeding insects, the whole body was weighed (g) to determine the effect of these compounds on *L. migratoria* growth.

Determination of Nutrition Indices and Food Intake Changes:

Feeding experiments were conducted according to Abu ElEla *et al.,* 2016 and Yuan *et al.,* 2020. Initial investigations focused on rutin concentrations ranging from 0.1 mM to 10 mM (0.1, 0.5, 2, 5, 10, 20, and 30 mM). Fifteen healthy fifthinstar nymphs of *L. migratoria* were starved for 24 h, weighed, and then fed a normal diet dipped in four concentrations of rutin: 5, 10, 20, and 30 mM. The diets were removed, and feces were collected. Each treatment concentration was replicated three times, and a parallel control of non-treated instars was also conducted. Following feeding trials, the nymphs, the discharged feces, and the remaining feed were weighed and dried at 80°C for 8 h until reaching a constant weight and weighed to determine mass loss from pre-feeding. Simultaneously, additional replicates were used to measure fresh weight and dry weight. Based on the nymph's water content, the dry weights of the pre-feeding nymphs and diet were calculated.

Nutritional indicators were calculated using standard gravimetric procedures described by Waldbauer (1968) as follows:

1) Consumption index (CI) measures the amount of food ingested per unit of time relative to the average weight of larvae over the feeding period, CI = C/[T)(A)]. Where C is the fresh weight of the leaf; T – the duration of feeding and A is the fresh weight of the insect during the feeding period.

2) Growth rate (GR) measures the amount of weight gained per unit of time relative to the average insect weight over the feeding period; GR = G / [T) (A)]. Where G; is the increase in the fresh weight of the insect.

3) Efficiency of conversion of ingested food into body tissues (ECI) is an overall measure of the ability of insects to use ingested food for growth, ECI = (G/C) x (100).

4) Efficiency of conversion of digested food into body tissues (ECD) is an overall measure of the ability of insects to use digested food for growth, ECD = [G/(C-F)] x (100). Where F; is the weight of feces during the feeding period.

5) Approximate digestibility (AD) measures the ability of insects to digest the introduced food, AD = [(C-F)/C] x (100).

Determination of Reproductive Activity and Developmental Indices:

The reproductive potential of *L. migratoria* was investigated by exposing the freshly emerged (0-1 day old) female adults treated at the fifth instar using the feeding

technique with 1 mM glycyrrhetinic acid, 10 mM rutin, and 30 mM quercetin during the pre-oviposition phase. Five replicates were conducted, each with five control insects and 25 experimental females. Females deposited their egg batches into sterile sand held in plastic cups (5 cm in diameter and 10 cm height). Egg deposition was monitored daily until the death of the females. The following reproductive parameters were recorded:

1) **The number of egg pods per female (NP\F)**: This measures the average number of egg pods lay by each female.
2) **Total of eggs per female (TNE\F)**: This measures the total number of eggs lay by each female.
3) **Weight of a sample of five eggs**: This provides an indication of egg size and development.
4) **Egg-laying rhythm (ELR)**: This describes the pattern of egg-laying over time.
5) **Pre-oviposition phase period (POP)**: This measures the time between adult emergence and the first egg-laying event.
6) **Fecundity rate**: This measures the total number of eggs laid per female.
7) **Fertility rate**: This measures the percentage of eggs that hatch.
8) **Hatching rate**: This measures the percentage of eggs that successfully hatch into nymphs.
9) **Sterility rate**: This measures the percentage reduction in fertility compared to the control group.

The remaining eggs of each pod were incubated in the sand and kept in darkness at 33°C for 17 days, as eggs typically hatch within 12-13 days. The sterility rate was also calculated by applying the following formula:

(% fertility in control - % fertility in treatment) / (% fertility in control) x100

Biochemical Analyses:

Preparation of Whole Homogenates For Analysis:

The whole body of all treated insects (nymph and adult) were homogenized in 20% (w/v) of either 20 mM Tris-HCl buffer, pH 8.0 containing 5mM β-mercaptoethanol for the determination of GSH levels and GST activity or in 50 mM potassium phosphate buffer, pH 7.0 for the determination of the other antioxidant enzymes. The homogenates were centrifuged at 11,000*xg* for 45 min. The supernatants were filtered through a plug of glass wool to remove floating lipids; the cytosolic fractions were termed as crude homogenates and stored at −20°C for further analyses.

Protein Determination:

Protein concentration was determined by the method of Bradford, 1976 using bovine serum albumin as a standard.

Glutathione Determination:

The total GSH was measured colorimetrically using the method of Saville, 1958. The cell homogenates were mixed with an equal volume of 13% *Trichloroacetic acid* (TCA). The precipitated proteins were removed by centrifugation at 2000 rpm for 10 min and the supernatant was used for the assay of total GSH.

Enzyme Assays:

Glutathione Transferase (GST):

Glutathione transferase activity (EC 2.5. 1.18) was determined according to the method described by Habig *et al.* (1974) by measuring the increase in the concentration of the conjugation product of GSH and 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm over 3 min at 30°C. One unit of GST activity is defined as the formation of 1 µmole product per min at 30°C.

Glutathione Peroxidase (GPx):

The activity of GPx (EC 1.11.1.9) was determined according to the method described by Paglia and Valentine (1967). The assay reaction mixture contained in1mL volume, 50 mM potassium phosphate buffer, pH 7.0, 0.005 M EDTA, 0.075 mM H_2O_2, 5.0 mM GSH, 0.28 mM NADPH, 1 IU GR, and a suitable crude enzyme homogenate volume. One unit is equivalent to the oxidation of 1 µmole of NADPH in 1 min, at 30°C. The extinction coefficient of NADPH was taken to be 6.22 $mM^{-1}cm^{-1}$.

Glutathione Reductase (GR):

The activity of GR (EC1.8.1.7) was determined spectrophotometrically at 30°C

following the decrease in absorbance at 340 nm according to the method described by Zanetti (1979). The assay reaction mixture contained a total volume of 1 mL, 50 mM potassium phosphate buffer, pH 7.0, 1 mM EDTA, 0.1 mM NADPH, 0.5 mM oxidized glutathione, and the enzyme solution. One unit of GR activity is defined as the amount of enzyme which oxidizes 1 µmole of NADPH per min.

Catalase (CAT):

Catalase (EC 1.11.1.6) activity was carried out according to the method described by Aebi (1984). The method is based on monitoring the rate of decomposition of H_2O_2 at 30°C. For CAT activity determination, a suitable volume of crude enzyme was added to 1 mL of substrate mixture, which consisted of 0.02 M H_2O_2 in 50 mM phosphate buffer, pH 7.0. The decomposition of H_2O_2 was followed by a decline in absorbance at 240 nm in 1 min. One unit of activity was defined as the calculated consumption of 1µmole of H_2O_2/min at 30 °C. The extinction coefficient of H_2O_2 was taken to be 43.6 M^{-1} cm^{-1}.

Statistical Analysis:

The Wilcoxon signed-rank test was used to compare the median levels of antioxidant enzymes between treatment and control groups. The Wilcoxon signed-rank test is a nonparametric test that was chosen due to the small data set and the data for some enzymes not being normally distributed. The p-value was calculated to detect the statistical significance among the treated and control groups. P-values less than 0.05 were considered statistically significant. The Shapiro-Wilk test was used to check the normality of the data sets. All statistical analyses were calculated using GraphPad Prism (version 5).

RESULTS

Feeding Effect on Body Weight of *L. migratoria* During Different Developmental Stages:

The effect of feeding glycyrrhetinic acid, rutin and quercetin starting at the beginning of the second stage on growth, development and survival from the second stage to adulthood was studied (Table 1). The three compounds studied significantly reduced the total body weight of the adult stage compared to control insects ($p<0.05$). Treatment with 10 mM rutin and 2 mM quercetin had a significant lower effect on the second instar and fifth instar ($p<0.05$) compared to control insects, Table 1. Feeding with any of the three compounds did not affect the survival rate of *L. migratoria* during the different growth stages.

Table 1: Effect of synthetic pure phenolic compounds glycyrrhetinic acid, rutin, and quercetin on whole body weight of *L. migratoria* during different developmental stages.

Feeding treatment	Second instar	Fifth instar	Adult Female
Control	0.31 ± 0.057	1.327 ± 0.36	1.66 ± 0.148
0.1 mM glycyrrhetinic acid	0.25 ± 0.031***	1.51 ± 0.18	1.05 ± 0.35***
1mM glycyrrhetinic acid	0.32 ± 0.053	1.35 ± 0.11	0.77 ± 0.029
5 mM rutin	0.26 ± 0.015	1.00 ± 0.21	0.73 ± 0.08
10 mM rutin	0.21 ± 0.025	1.31 ± 0.34	0.79 ± 0.123
2 mM quercetin	0.14 ± 0.013*	0.99 ± 0.19*	1.367 ± 0.035*
20 mM quercetin	0.24 ± 0.049	1.177 ± 0.063***	0.87 ±0.13

Values are presented as mean ± SD.

**P values ≤ 0.05 are considered significant when using the one sample t-test.*

**** p = 0.0001, * p = 0.012.*

All data set for each stage showed normal distribution by using Shapiro-Wilk test.

Food Intake Changes and Nutritional Indices:

The results presented in Figure 2, showed that rutin had highly significant effects on the conversion efficiency of digested food into body substance % (ECD%), consumption index (CI), and relative growth rate (GR) after increasing the concentration of rutin from 5 to 30 mM (P = 0.0001 Approximate digestibility (AD%) values significantly decreased from 10 to 30 mM rutin concentrations, along with a decline in ECI% values.

Biochemical Changes:

Feeding Effect on Total GSH Concentration and Antioxidant Enzyme Activities:

During normal development under natural feeding conditions, the GSH concentration in *L. migratoria* exhibited a significant decrease from 0.4 µmol/mg protein in the second stage to 0.25 µmol/mg protein in the fifth stage, ultimately reaching 0.116 µmol/mg protein in the adult stage. The activities of GPx, GST, and CAT enzymes decreased significantly from the second instar to the adult (Tables 2, 3 and 4).

The results showed that after feeding glycyrrhetinic acid, rutin, and quercetin; the three compounds had a significant effect on GSH levels, detoxification, and antioxidant enzyme activities in the adult stage of *L. migratoria* as shown in Table 4. The effect of the three studied compounds on the second stage of *L. migratoria* was weak, except for 5 mM rutin, which led to a decrease in the activity of GPx and CAT enzymes (Table 2).

Biochemical Changes to Feeding Glycyrrhetinic Acid:

Compared to control insects, feeding 0.1 mM glycyrrhetinic acid in the second stage significantly decreased GSH concentration to 62 % of its concentration in control insects (Table 2). However, no significant changes were observed in the fifth and adult stages (Tables 3&4). Feeding insects with a higher concentration of glycyrrhetinic acid (1 mM) significantly increased the level of both GSH (166%) and CAT activity (202%) at the fifth stages (Table 3). However, after feeding with 0.1 mM glycyrrhetinic acid, GST activity was significantly increased in the fifth stage (2-fold) and adult (3-fold) compared to controls (Tables 3&4). The antioxidant enzyme CAT was also significantly increased in the fifth stage (2.4-fold) and then to 1.4-fold of control activity in the adult stage. The CAT activity was significantly increased in the adult stage (194%) with feeding 1 mM glycyrrhetinic acid when compared to controls (Table 4).

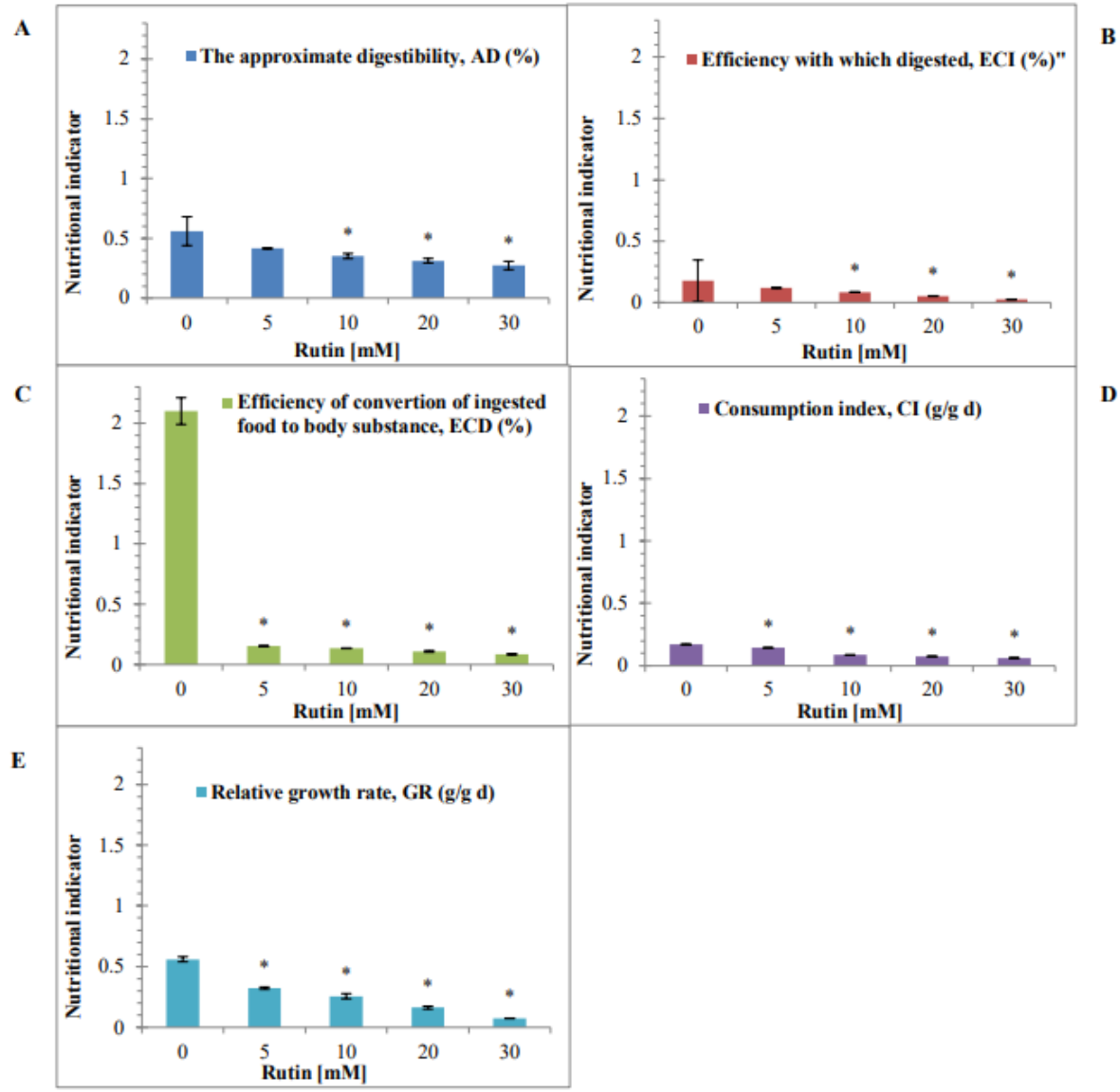


Fig. 2: Changes in food intake of *L. migratoria* fifth stage fed with different rutin concentrations. All the nutrition indicators were calculated based on dry weight. Values are presented as mean ± SD. *P values ≤ 0.05 are considered as significant by using the One sample t-test. Statistical analysis indicated that one sample t-test results were significant for 5, 10, 20mM rutin concentrations. However, the concentration of 30mM rutin did not show a statistically significant effect compared to control group.

Biochemical Changes To Feeding Rutin:

The results indicated that feeding second stage *L. migratoria* with 5 mM rutin significantly decreased the activity of both GPx and CAT by 62% and 56%, respectively, of their enzymatic activity in the control insects (Table 2**).** The same activity decrease of such enzymes was observed by using 10 mM rutin. Feeding insects with 10 mM rutin also decreased the level of GSH to less than 65% in the second stage. Levels of GSH and the activities of GPx, GST, and CAT in the adult stage significantly increased compared to their activity in control insects after feeding with both 5 and 10 mM rutin (Table 4).

Biochemical Changes to Feeding Quercetin:

Feeding the second stage of *L. migratoria* with 2 mM quercetin significantly increased the level of GSH (204%) compared to its level in control insects. In contrast, the activity of GST decreased to 59.5%compared to controls (Table 2). By increasing the insect

feed concentration to 30 mM, all biochemical aspects examined increased in the fifth stage and adults except GR activity decreased to 52.5 % in the adults compared to their values in the control insects (Tables 3&4).

Table 2: Effect of synthetic pure phenolic compounds glycyrrhetinic acid, rutin, and quercetin on total GSH concentration and antioxidant enzymes of *L. migratoria* in the second stage of development.

Treatments	GSH	GPx	GR	GST	CAT
	µmol/mg protein	*µmol /min/ mg protein*			*nmol /min/ mg protein*
control	0.402±0.05	0.184±0.023	0.125±0.037	0.89±0.30	1.47±0.25
0.1 mM glycyrrhetinic acid	0.250±0.028*	0.144±0.035	0.112±0.028*	0.704±0.052	1.74±0.25
1 mM glycyrrhetinic acid	0.362±0.018	0.159±0.026	0.130±0.043	0.516±0.105	1.35±0.05
5 mM rutin	0.375±0.082	0.114±0.035*	0.262±0.111	0.710±0.223	0.83±0.052*
10 mM rutin	0.259±0.069*	0.150±0.031	0.188±0.039	0.818±0.228	1.35±0.041
2 mM quercetin	0.830±0.022*	0.15±0.028*	0.11±0.06	0.53±0.08*	1.35±0.16
30 mM quercetin	0.427±0.044*	0.184±0.052	0.159±0.043	0.817±0.060	0.83±0.108

Values are presented as mean ± SD.
**P values ≤ 0.05 are considered significant compared to control group using the Shapiro-Wilk-rank test.*
P =0.0156 for GSH versus GPx, and GR.
P = 0.046 for GSH versus GST.
GSH, reduced glutathione; GST, glutathione transferase; GPx, glutathione peroxidase; GR, glutathione reductase; CAT, catalase

Table 3: Effect of synthetic pure phenolic compounds glycyrrhetinic acid, rutin, and quercetin on total GSH concentration and antioxidant enzymes of *L. migratoria* in the fifth stage of development.

Treatments	GSH	GPx	GR	GST	CAT
	µmol/mg protein	*µmol /min/ mg protein*			*nmol /min/ mg protein*
control	0.25±0.06	0.13±0.009	0.099±0.002	0.69±0.17	0.63±0.15
0.1 mM glycyrrhetinic acid	0.23±0.066	0.16±0.011*	0.13±0.031	1.49±0.313*	1.53±0.05*
1 mM glycyrrhetinic acid	0.43±0.053*	0.16±0.027	0.14±0.025	0.94±0.081	1.27±0.08*
5 mM rutin	0.38±0.082	0.11±0.035	0.26±0.111	0.71±0.223	0.83±0.052
10 mM rutin	0.20±0.006	0.10±0.013	0.09±0.022	0.80±0.088	0.45±0.285
2 mM quercetin	0.16±0.004	0.20±0.018*	0.08±0.0094*	0.53±0.018	0.50±0.021
30 mM quercetin	0.43±0.044*	0.18±0.052	0.16±0.043	0.82±0.060	0.83±0.108

Values are presented as mean ± SD.
**P values ≤ 0.05 are considered significant compared to control group using the Shapiro-Wilk-rank test.*
P =0.0156 for GSH versus GR, GST, CAT.
P = 0.0313 for GSH versus GPx.
GSH, reduced glutathione; GST, glutathione S-transferase; GPx, glutathione peroxidase; GR, glutathione reductase; CAT, catalase

Table 4: Effect of synthetic pure phenolic compounds glycyrrhetinic acid, rutin, and quercetin on total GSH concentration and antioxidant enzymes of *L. migratoria* in the adult stage of development.

Treatments	GSH	GPx	GR	GST	CAT
	µmol/mg protein	*µmol /min/ mg protein*			*nmol /min/ mg protein*
Control	0.116±0.006	0.062±0.012	0.120±0.009	0.343±0.018	0.335±0.038
0.1 mM glycyrrhetinic acid	0.231±0.023*	0.123±0.013*	0.081±0.011*	1.021±0.329*	0.472±0.08*
1 mM glycyrrhetinic acid	0.198±0.003*	0.095±0.007*	0.070±0.006*	0.880±0.228*	0.650±0.06*
5 mM rutin	0.186±0.03*	0.094±0.015*	0.072±0.002*	0.611±0.135*	0.637±0.080*
10 mM rutin	0.165±0.011*	0.079±0.021	0.072±0.002	0.570±0.107*	0.474±0.057*
2 mM quercetin	0.162±0.002*	0.108±0.01*	0.023±0.004*	0.315±0.021	0.490±0.007*
30 mM quercetin	0.229±0.01*	0.088±0.014	0.063±0.002*	0.650±0.103*	0.444±0.017*

Values are presented as mean ± SD.
**P values ≤ 0.05 are considered significant compared to control group using the Shapiro-Wilk-rank test.*
P =0.0012 for GSH versus GPx.
P =0.0156 for GSH versus GST and CAT.
P = 0.0313 for GSH versus GR.
GSH, reduced glutathione; GST, glutathione S-transferase; GPx, glutathione peroxidase; GR, glutathione reductase; CAT, catalase.

Changes in the Number and Quality of Eggs and Reproductive Activity of The Adult Female *L. migratoria*:

The effect of feeding glycyrrhetinic acid, rutin, and quercetin on egg number and quality and the reproductive activity of adult female *L. migratoria* were investigated after feeding the second stage with high concentrations of each compound **(**Fig. 3). The control group exhibited a pre-oviposition period (POP) of 10.7 days, which increased gradually with three compounds, reaching 18.9 days for the highest concentration of 30 mM quercetin. Similarly, the egg-laying rhythm (ELR) extended from 3.4 days in the control to 8.9 days with 30 mM quercetin. The number of pods per female (NP/F) and the total number of eggs per female (TNE/F) decreased among three compounds, with quercetin showing the most significant reduction to 1.4 pods and 47.9 eggs. Fecundity and fertility percentages also decreased with quercetin-treated females exhibiting only 31.7% fecundity and 33.3% fertility, respectively. The corrected sterility rate (CS %) showed the highest sterility in the quercetin group at 61.1%.

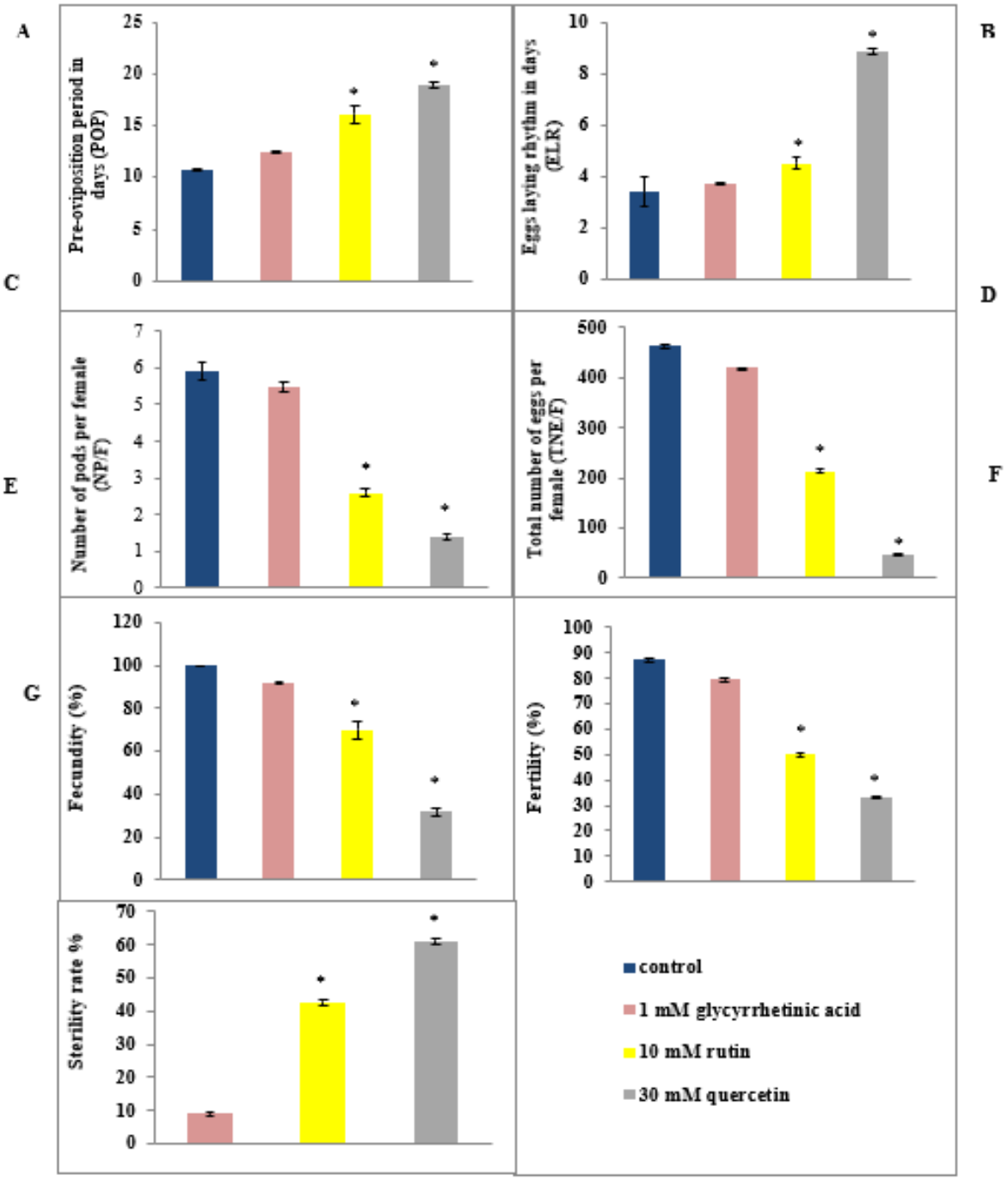


Fig. 3: Changes in the number and quality of eggs and reproductive activity of the adult female L. migratoria feeding with 1 mM glycyrrhetinic acid, 10 mM rutin, and 30 mM quercetin. A) POP: pre- oviposition period; B) ELR: Eggs laying rhythm; C) NP/F: Number of pods per female; D) TNE/F: Total number of eggs per female; E) Fecundity %; F) Fertility % and G) CS: sterility rate. Values are presented as mean ± SD of three independent experiments. *P values ≤ 0.05 are considered significant compared to control group using the Shapiro-Wilk-rank test.

DISCUSSION

The present study showed significant reductions in the whole-body weight of *L. migratoria* adult stages when compared to the control group. These reductions were seen across all investigated concentrations of glycyrrhetinic acid, rutin, and quercetin. The weight loss in adult insects suggests that these

substances have an impact on dietary intake and development, possibly due to their accumulation in insect tissues. This finding aligns with previous research by Tu *et al.* (2012), who reported that feeding damage primarily affects the last instars and adults, with the early instars being less affected.

The effectiveness of glycyrrhizin or glycyrrhetinic acid against grasshoppers and locusts is not widely known. However, studies have validated the cytotoxic properties of glycyrrhizin (Batiha *et al.* 2020; Nascimento and de Araújo, 2022). Rutin and its aglycone quercetin are well-known plant flavonoids that play a role in the plant-insect relationship by acting as resistance mechanisms in plants. However, the effects of these compounds on insect behavior can vary depending on the insect and plant species involved.

For example, rutin has been found to be toxic to the woolly apple insect, *Eriosoma lanigerum* (Hemiptera: Pemphigidae). It has also been observed that the cockatiel *Melolontha Melolontha* (Coleoptera: Scarabaeidae) had a high mortality rate after feeding on Quercus robur (Fagaceae) oak leaves sprayed with a rutin solution. On the other hand, quercetin had no effect on the behavior and growth of the same insect species (Stec *et al.*, 2021). Rutin has also shown deleterious effects on insects such as *Lymantria dispar*, *Spodoptera litura*, *Pectinophora gossypiella*, *Heliothis virescens*, *Spodoptera eridania*, and *Helicoverpa zea* (Huang *et al.*, 2020).

A diet containing quercetin, on the other hand, has been found to inhibit the growth of common tomato pests such as *Helicoverpa armigera* (Lepidoptera: Noctuidae) and the beet armyworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). It has also been shown to reduce the pupation rate of *H. armigera* and *S. litura*. Additionally, quercetin has been found to hinder the growth and development of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) larvae through cumulative toxic effects resulting from quercetin accumulation. Quercetin may serve as an effective botanical pesticide against various polyphagous pests, as has been shown to inhibit *E. lanigerum* survival. Furthermore, the amount of quercetin added to the diet of grasshoppers (*Oedaleus asiaticus*) larvae has been strongly associated with mortality. Quercetin has also been shown to increase the death rate of the autumn armyworm larvae, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), the gipsy moth (Lepidoptera: Lymantriidae), and *Oedaleus asiaticus* Bey-Bienko (Orthoptera: Acrididae) (Gao *et al.;* 2022).

These examples illustrate the varying effects of rutin and quercetin on different insect species. While these compounds can be effective against certain types of insects, their impact may differ when exposed to other species. Thus, it is evident that the effects of these compounds can vary depending on the specific insect species and their characteristics.

From a mortality perspective, the three examined compounds did not influence insect survival. However, we observed that insects displayed a refusal to eat the concentrations of rutin used in this study for a certain period. This result may indicate a repelling effect of rutin, which caused the insects to stop consuming the food treated.

In our study, when rutin was added to the normal diet of *L. migratoria*fifth stage, we observed a significant reduction in ECD (ecdysis, or molting), CI (developmental duration), and GR (growth rate) compared to the control groups. These findings are consistent with previous research that reported similar decreases in CI and GR values for *S. litura* (Fabrius) (Lepidoptera: Noctuidae) when fed a neem-based diet. Additionally, a decrease in GR has been observed in the larvae of the red palm weevil following a diet containing coumarin. Similar results were observed when a high concentration of tannic acid (3%) was added to the diet of 4th instar *H. cunea* larvae, resulting in lower ECI (efficiency of conversion of ingested food) and ECD (efficiency of conversion of digested food). Furthermore, the ECI of *S. litura* larvae fed with sesame was reduced. These examples

demonstrate that various secondary plant metabolites can lead to reduced feeding and growth parameters in different insect species, suggesting that more food is metabolized for energy and less food is converted into body weight (Yuan *et al.,* 2020).

In this investigation, we observed a decrease in feeding indices values as the feeding concentration of rutin increased from 5 to 30 mM. This decrease in feeding indices was accompanied by a reduction in total body weight at different developmental stages, starting from the second instar to the adult stage, after treating the insects with 5 and 10 mM rutin. These results suggest that rutin treatment has a negative impact on the biomass of L. migratoria, as evidenced by a decrease in the overall body weight gain in adult insects. Furthermore, the significant decrease in %ECD in insects treated with rutin, starting from 5 mM concentration, indicates that feeding with rutin affects food utilization efficiency. This implies that a significant portion of the ingested food is utilized to overcome metabolic issues induced by rutin, rather than supporting growth.

Insects are exposed to various environmental stresses, including oxidative stress, which is particularly pronounced in herbivorous insects due to their high intake of plant phenols. The elimination of phenols can lead to increased generation of oxidative radicals and oxidative stress. Certain phenolic compounds can exhibit pro-oxidant activity, especially at high acidic pH concentrations and in the presence of transition metals. This pro-oxidant activity can result in the production of ROS or the inhibition of antioxidant systems such as GST. For example, flavonoids like quercetin, delphinidin, myricetin, and quercetagetin have been found to generate ROS during auto-oxidation, affecting insect mitochondrial respiration in vitro (Hamed *et al.,* 2019). The accumulation of these pro-oxidants and redox allelochemicals in the insect body can lead to lipid peroxidation in cell membranes and direct damage to biomolecules such as DNA, thereby disrupting cellular metabolic processes (Silva *et al.*, 2021; Guneidy *et al.,* 2022).

Oxidative stress is also implicated as a mechanism for the toxic effects of pesticides, particularly when insects are exposed to low doses over extended periods. This occurs due to an imbalance between the body's antioxidant defence s and the production of free radicals or ROS. Oxidative stress occurs when the level of free radicals exceeds the capacity of antioxidant defence mechanisms (Mossa et al., 2018). Phytophagous insects allocate a significant amount of energy to detoxifying plant phenolic chemicals by modulating the activity of antioxidant and detoxification enzymes, making these phenolic compounds less toxic and facilitating their transport or excretion (Gao *et al.,* 2022).

Overall, our findings suggest that rutin treatment affects feeding indices, body weight, and food utilization efficiency in *L. migratoria*. These effects may be attributed to the pro-oxidant activity of rutin and its impact on oxidative stress in insects. Understanding the physiological and metabolic responses of insects to plant secondary metabolites like rutin can provide valuable insights into their potential as pest management agents.

Antioxidant enzymes play a crucial role in combining the accumulation of oxidative radicals in insect tissues throughout the insect's lifespan, thereby influencing insect development. Disruption of this integrated antioxidant system can alter its function, depriving pests of their defence mechanism and potentially serving as a key aspect of pest control (Fahmy *et al.,* 2022).

A functional antioxidant system relies on the collaboration of various specialized systems and molecules, including enzymes and non-enzymatic antioxidants with low molecular weight. Non-enzymatic components such as glutathione, ascorbic acid, vitamin E, uric acid, and thioredoxin are important in this regard. These low molecular weight antioxidants interact with ROS in a less specific manner compared to enzymes, allowing them to exert their activity more comprehensively (Dmochowska-Ślęzak *et al.,* 2015). Among the enzymes involved,

CAT and GST are particularly notable as front-line antioxidant defence enzymes against oxidative conditions (Dampc *et al.*, 2020; Fahmy *et al.*, 2022). Catalase converts hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2) to counteract oxidative radicals, while GST is responsible for cellular detoxification of xenobiotic substances by eliminating H_2O_2 from cells through the conjugation of glutathione (GSH) with electrophilic molecules, which is an early response to pollutant-induced stress (Silva e*t al.*, 2021; Fahmy *et al.*, 2022).

Additionally, rutin has been found to interact with certain enzymes, including digestive enzymes, primarily through the C-H and O-H bonds in their glycoside structure, resulting in steric hindrance and affecting their activities. This effect was observed to be more limited in quercetin (Wang *et al.*, 2022).

In our study, we observed a decrease in the levels of GSH and related antioxidant enzymes, particularly GST and CAT, during the normal development of *L. migratoria* under normal feeding conditions. This decrease was evident from the second stage and persisted until adulthood.

However, when *L. migratoria* insects were fed with the three compounds under investigation (glycyrrhetinic acid, rutin, and quercetin) at different stages (second, 4th, fifth instar, and adult), we observed varying impacts on the activity levels of the examined enzymes. The fifth instar stage showed the most significant impact, followed by the adult stage.

These findings suggest that the antioxidant activity and detoxification processes undergo variations during the development of *L. migratoria*. The increased activity of the tested enzymes during the early stages of growth, followed by a decline upon reaching adulthood, indicates dynamic regulation of antioxidant defense systems. It is possible that the weaker behavioral systems of nymphs, compared to later stages and adults, require higher antioxidant enzyme activity to provide protection and support their continued growth until maturity.

Similar findings regarding normal development and changes in the antioxidant system were reported in the study by Fahmy *et al.* (2022). The study observed that various cellular events occur during normal development to enhance the antioxidant system. It was found that the early stages of insect development exhibited the highest metabolic rate compared to later stages. This higher metabolic rate in the early stages is associated with increased oxygen consumption and a higher feeding rate. However, as the insect ages, metabolic efficiency decreases. This decrease in metabolic efficiency may be attributed to factors such as reduced feeding rate, changes in energy allocation, and alterations in physiological processes during development.

In our study, we observed a significant increase in GSH levels and antioxidant enzyme activities following the feeding of *L. migratoria* insects with glycyrrhetinic acid, rutin, and quercetin. This increase was particularly evident during the adult stage of development, with a notable induction of GST and CAT activity compared to the control insects. Interestingly, the three compounds did not have any significant influence on insect mortality or survival rates.

Our findings are consistent with those of Fahmy *et al.* (2022), who reported similar observations in the cotton leaf worm (*S. littoralis*). In their study, they observed higher activities of GST, CAT, and phenoloxidase during the late larval stages, specifically the fifth and sixth stages, compared to the early stages. This increase in enzyme activity was attributed to the extensive consumption of chlorogenic acid and tannin present in the host plant's leaves, which may generate oxidative radicals and lead to the accumulation of ROS.

In our study, feeding *L. migratoria* insects with the three compounds, glycyrrhetinic acid, rutin, and quercetin, had significant effects on all the reproductive indicators examined. Quercetin exhibited the highest effect, while glycyrrhetinic acid had a slight effect. Both rutin and quercetin significantly reduced fecundity percentage, fertility percentage, and sterility rate (CS

percentage) compared to the control group. Moreover, higher concentrations of the three compounds significantly decreased the total weight of the fed insects by approximately 50% compared to the control body weight. The compounds also affected the total number of eggs per female (TNE/F), with quercetin at 30 mM resulting in 214 eggs and rutin at 10 mM resulting in 47.9 eggs, compared to 463.7 eggs in the control group.

Egg size plays a crucial role in offspring reproductive success and maternal inheritance. Larger eggs are more resilient to environmental challenges such as larval competition, starvation, and nutritional stress. Conversely, smaller eggs hatch faster but have lower survival rates. Egg production, as a measure of female fertility, is influenced by individual body size and lifespan, affecting both the total number of eggs produced and the number of eggs in a single egg pod (Hu *et al.*, 2022).

According to Abdellaoui *et al.* (2018), the high polyphenol content of olive leaf extract (OLE) contributes to the occurrence of several reproductive abnormalities in adult female *L. migratoria*. This could be due to the anti-nutritional action of phenolic compounds. Oleuropein, the major phenolic compound found in olive leaf extracts, exhibits potent chemical repellent properties. It has been shown to be highly effective against the female olive fruit fly (*Dacus oleae*) as a strong chemical repellent. The anti-feeding activities of OLE against other insect orders, such as *S. gregaria* and *S. littoralis*, have also been documented, leading to a reduction in food intake rates. The composition of OLE exerts an inhibitory effect on phago-stimulant receptors, impacting the feeding capacity of many insects by reducing the relative growth rate, the efficiency of conversion of ingested food values, and the efficiency of conversion of digested food values. The anti-feeding abilities of polyphenols may be attributed to their ability to inhibit digestive proteases and hydrolases, including amylases, as well as polyphenol oxidase (Melzig, 2023). This increases leaf stiffness, which hampers herbivore feeding and diminishes the nutritional value of the leaf. Additionally, it interferes with protein digestion in herbivores, leading to decreased insect growth and development.

In conclusion, this study highlights the strong protective defence system and high adaptability of *L. migratoria*, as evidenced by the absence of any effect on insect survival. The key findings can be summarized as follows: First, the three substances examined - glycyrrhetinic acid, rutin, and quercetin - induced significant changes in glutathione (GSH) levels and antioxidant enzyme activity, particularly during the adult stage of development. Second, rutin and quercetin demonstrated a notable negative impact on the reproductive capacity of adult females, resulting in a significant reduction in fecundity and fertility. Third, rutin consumption led to observed alterations in food intake and nutritional indices. These findings suggest that these compounds have the potential to modulate the insect's antioxidant defence system and can interfere with insect development and reproduction, making them promising candidates for pest management purposes.

However, it is important to note that this study represents the first investigation into the effects of these compounds on *L. migratoria* at different developmental stages. Further research on a larger number of insects is necessary to explore the possibility of increasing concentration and extending the exposure time of these substances. This is important to confirm their effectiveness in controlling migratory locusts and preventing their proliferation.

Declarations:

Ethical Approval: Not applicable.

Conflict of interests: The authors declare no conflicts of interest.

Authors Contributions: R.A.Guneidy: Developing the research idea and work plan, collecting scientific material, writing and interpreting the results, writing the research paper, reviewing the research, and overseeing the publication process. Z.Fathy: Obtaining insects, feeding them with the studied

compounds, conducting experiments related to insect physiology, and reviewing the research. A.Shokeer: Performing statistical analysis of the results, analyzing the findings, and reviewing the research. E.R.Zaki & G.S.A.Karim: Preparing materials, conducting the practical portion, writing and analyzing the results, and reviewing the research.

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