Journal of Entomology

ISSN 1812-5670

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Entomology

ISSN 1812-5670
DOI: 10.3923/je.2022.9.19

Research Article
Antioxidant Defense of *Apis mellifera* L. in Response to Chlorophyllin Derivatives: As a Marker of Ecotoxicological Stress

[1]Samar Mamdouh Nassar, [2]Salwa Ibrahim Sebak, [1]Tarek Al-Tayb and [3]Azza Mohamed Elgendy

[1]National Institute for Laser Enhanced Sciences, Cairo University, Cairo, Egypt
[2]Department of Zoology, Faculty of Science, South Valley University, Qena, Egypt
[3]Department of Entomology, Faculty of Science, Cairo University, Cairo, Egypt

Abstract

Background and Objective: Chlorophyllin derivatives, mammal-safe pesticides, are using recently to control many agricultural and medical pests such as lepidopteran and mosquitoes population. The objective of this work was to assess the possible environmental risks for the chlorophyllin derivatives field application, particularly related to pollinating insect, *Apis mellifera*. **Materials and Methods:** In this study, the foraging worker honey bees fed on tri-sodium copper and magnesium chlorophyllin LC_{50} (10^{-5} and 3×10^{-3} M L^{-1}, respectively) were used to measure the total antioxidant capacity, superoxide dismutase and glutathione S-transferase. Also, midgut cells were papered for the comet assay. **Results:** The tri-sodium Cu-chlorophyllin increased the TAC, SOD and GST activities in the body homogenate of honey bees in response to the oxidative stress of both chlorophyll and Cu elements. **Conclusion:** Furthermore, the results of the genotoxicity experiment revealed that Cu-chlorophyllin caused DNA damage in the gut cells more than Mg-chlorophyllin and its effect might be due to copper elements.

Key words: Antioxidant enzyme, *Apis mellifera*, chlorophyllin derivatives, DNA damage, GST, Cu-chlorophyllin, superoxide dismutase

Citation: Nassar, S.M., S.I. Sebak, T. Al-Tayb and A.M. Elgendy, 2022. Antioxidant defense of *Apis mellifera* L. in response to chlorophyllin derivatives: As a marker of ecotoxicological stress. J. Entomol., 19: 9-19.

Corresponding Author: Azza Mohamed Elgendy, Department of Entomology, Faculty of Science, Cairo University, Cairo, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Honey bees are the most important species that pollinate flowering plants and among a few species that are domesticated by humans for agricultural and industrial economic purposes[1]. Honey bee, *Apis mellifera* has a major role in sustaining food security and maintaining biodiversity for the natural ecosystems [2]. This important insect faces many complex stressors: Biological include pathogens, parasite and microorganisms[3-5], environmental include: Temperature and humidity[6], chemicals include: Metal and metalloid contaminants[7,8], different types of plant protection products such as insecticides, acaricides, herbicides and fungicides have applied to control agricultural pests[9]. In addition, the anthropogenic activities associated with urbanization and industrialization has created localized and regional pollution problems[10]. A lot of publications have studied the sub-lethal and accumulation effects of many natural and synthetic pesticides groups on honey bee life such as fitness, foraging activity, flight activity, sensorial ability, neurotoxicity, detoxification, metabolism and oxidative stress[11]. Less study focused on enzymatic and molecular responses of honey bees using genomic, metabolomic and transcriptomic techniques and biomarkers[12].

The detoxification systems of insects, especially honey bees, respond quickly to chemical and biological stresses[13,14]. In honey bees, oxidative stress due to different biotic and biotic factors was examined in many kinds of literature and the activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione s-transferase (GST), glutathione peroxidase (GPx), peroxidase (POD)[15-17]. Chlorophyllin derivatives have a new mode of action on insects and consider environmentally safe compounds.

This photoactive compound is accumulated inside the insect body and upon exposure to sunlight, the reactive oxygen species (ROS) is produced that cause cellular and molecular destruction[18-20]. Copper chlorophyllin has been added safely as animal nutrition[21]. The reduction of mosquitos' population in endemic areas using chlorophyllin derivatives have been recorded[22,23]. Afify *et al.*[24] showed that Cu and Mg-chlorophyllin caused death to *Culex pipiens* larvae due to physical damage of tissues and direct effect on their biochemical and physiological parameters. Recently, Nassar *et al.*[25] have studied the toxicity effect of both copper and magnesium tri-sodium chlorophyllin on forage honey bee's *A. mellifera* L., in laboratory and semi-field conditions. The LC_{50} of Cu and Mg-chlorophyllin measured in the laboratory were 10^{-5} and 10^{-3} M L^{-1}, respectively. Few studies on the cytotoxicity and genotoxicity impact of pesticides (insecticides, fungicides and acaricides) on honey bees have been done[12,26,27]. In the current work, the biochemical effects, total antioxidant capacity TAC, SOD and GST enzyme activities, were measured in the total body homogenates of forage bees exposed to LC_{50} of Cu and Mg chlorophyllin for 3 days in light and dark conditions. Furthermore, the genotoxic impact was estimated as DNA damage using Comet assay. This work presented the biochemical responses of honey bees to human safe pesticides, chlorophyllin derivatives which are crucial for their implementation to control pests without affecting the beneficial pollinators in fields worldwide.

MATERIALS AND METHODS

Honey bee sampling: Laboratory experiments were carried out with honey bee foragers of *Apis mellifera* L. (Hymenoptera: Apidae) from December, 2015 to October, 2018. The adult workers were obtained from the Apiculture station, Faculty of Agriculture, Cairo University, Giza, Egypt, where honey bee colonies were maintained according to the standard commercial technique in the field. For this study, foraging bees were used when they start performing external tasks[28,29]. Based on farming records, no obvious diseases were observed on units or colonies and pesticides free. According to Nassar *et al.*[25] the foraging workers were collected from the front of the hives in a plastic bag then transferred into experimental foam containers (10×7×12 cm) and left overnight before treatment. The bees were fed a 50% (w/v) sucrose solution with or without the chlorophyllin derivatives *ad libitum* at 25±2°C with 65±5% RH, a photoperiod of 8:16 (light: dark) for control light (CL) and treated light group (TL). The third group was fed on sucrose solution with chlorophyllin derivatives and kept in dark conditions (TD). The used LC_{50} of Cu-chlorophyllin and Mg-chlorophyllin were 10^{-5} and 3×10^{-3} M L^{-1}, respectively. The fourth group of bees were collected from the field (FC). Bees were collected at the end of the first, second and third day of the feeding. The bodies of five honey bees were homogenized in 500 µL ice-cold phosphate buffer saline (pH 7.4). The homogenates were centrifuged at 10,000 g for 15 min at 4°C and the supernatant was stored at -20°C in the freezer till use for further analyses. Total antioxidant capacity (TAC), superoxide dismutase (SOD) and glutathione S-transferase (GST), as well as protein content, were assayed in bee body homogenate.

Antioxidant enzyme assay: Total antioxidant capacity (TAC) was assayed according to Koracevic *et al.*[30]. TAC was determined using a commercial antioxidant colourimetric assay kit (Biodiagnostic and research reagent, Egypt). The absorbance read against blank at 505 nm and total antioxidant concentration results were expressed in mM L^{-1}.

Superoxide dismutase (SOD): Superoxide dismutase (SOD) has been assayed according to Nishikimi *et al.*[31]. The total SOD from cytosol, mitochondria and extracellular spaces inhibit the phenazine methosulphate-mediated (PMS) reduction of nitroblue tetrazolium dye (NBT). The SOD was measured as the increase in absorbance at 560 nm. The SOD activity can be expressed as a function of the protein (U mg^{-1} protein).

Glutathione S-transferase (GST): Glutathione S-transferase (GST) activity was determined using Habig *et al.*[32] methods. Total GST activity (cytosolic and microsomal) kit (Biodiagnostic and research reagent, Egypt) has been used. The conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione is increased by an increase in absorbance at 340 nm and is directly proportional to the GST activity in the sample. Enzyme activity was converted into the number of units (U) per 1 mg of protein.

The proteins were assayed using Biuret reagent (Biodiagnostic and research reagent, Egypt) at a wavelength of 550 nm[33]. Simply, protein produces a violet colour with Biuret reagent, alkaline cupric sulfate and the intensity of which is proportional to their concentrations.

Detection of DNA damage: The comet assay was used to analyze the percent of DNA damage in the gut cells of worker honey bees after feeding on Cu-chlorophyllin ($LC_{50} = 10^{-5}$ M L^{-1}) or Mg-chlorophyllin ($LC_{50} = 3 \times 10^{-3}$ M L^{-1}) and exposure to sunlight for 3 days (8 hrs per day). Honey bees fed on 50% sucrose solution and exposure to sunlight was used as control. Briefly, the guts of three honey bees for each treatment were maintained with 200 µL of PBS and centrifuged at 1000 rpm for 10 min. Then, 90 µL of low melting agarose was added to 10 µL of isolated cells and loaded on microscope slides, pre-coated with 1.5% normal melting point agarose (NMA). Next, covered the slide and then slides were coaled on ice. After agarose solidified, we removed the cover slips and slides were immersed in a lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 0.25 M NaOH, 1% TritonX-100 and 10% dimethylsulfoxide (DMSO), pH 10.0) for 24 hrs at 4°C. After lysis, slides were placed in a horizontal gel electrophoresis tank and DNA was allowed to unwind for 20 min in electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH 13). Electrophoresis was carried out at 24 V and 270 mA, at 4°C, for 20 min. Then neutralize the samples in 0.4 M Tris–HCl (pH 7.4), fixed with methanol and allowed to dry overnight at room temperature before staining with ethidium bromide (2 µg mL^{-1}). Comets were analyzed with an Axio fluorescence microscope (Carl Zeiss, Germany) with an excitation filter of 524 nm and a barrier filter of 605 nm. Three replicates were prepared and each of them consisted of a pool of 3 guts. The most common parameters analyzed were the percentage DNA in the tail (DNA%), tail moment and tail length[34].

Statistical analysis: Averages of the measurements on multiple aliquots of each sample for each treatment were used as the data set for the analysis. Statistical significance of differences among activities of TAC, SOD and GST level was calculated with two-way ANOVA. Subsequently, means were separated by Tukey's HSD test (a = 0.05).

RESULTS

Antioxidant protection (TAC, SOD and GST activities): The TAC, SOD and GST activities showed a significant increase in the body homogenate of bees fed on Tri-sodium Cu-chlorophyllin and exposed to light compared to that fed on tri-sodium Mg-chlorophyllin under the same experimental conditions.

Total antioxidant capacity (TAC): The bees fed on Cu-chlorophyllin showed a significant increase of the measured TAC in the first, second and third day of feeding under light (TL: 3.59±0.01, 3.36±0.02 and 2.82±0.2 mM L^{-1}, respectively) and dark condition (TD: 2.37±0.03, 2.55±0.09 and 2.47±0.00 mM L^{-1}, respectively) Table 1 and Fig. 1a. Furthermore, FC bees little increase of TAC compared to the control light group, significant difference, $p<0.01$ (Table 1 and Fig. 1b). On the other hand, TAC measured in body homogenates of Mg-chlorophyllin fed bees have no significant increase compared to the CL group and were nearly the same TAC measured in the FC group. (Supplemented Table 1).

SOD activity: The highest SOD activities have been measured in the bees fed on Cu-chlorophyllin and exposed to light (0.43, 0.32 and 0.41 U μg^{-1} protein) Table 1 and Fig. 2. Then it was followed by SOD activities of the dark group and finally, the field-collected group (Table 1 and Fig. 2a). The SOD activity measured in the body homogenate of the Mg-chlorophyllin fed bees was almost the same in TL (0.28, 0.27 and 0.28 U μg^{-1} protein) and TD (0.25, 0.26 and 0.27 U μg^{-1} protein) Table 1 and Fig. 2b and Supplemented Table 2.

GST activity: Cu- chlorophyllin fed group exposed to light, TL, showed the highest GST activities (2.4, 3.07 and 2.56 U μg^{-1} protein) Table 1 and Fig. 3a. The GST activities measured from FC bees were observed to be higher than that measured from

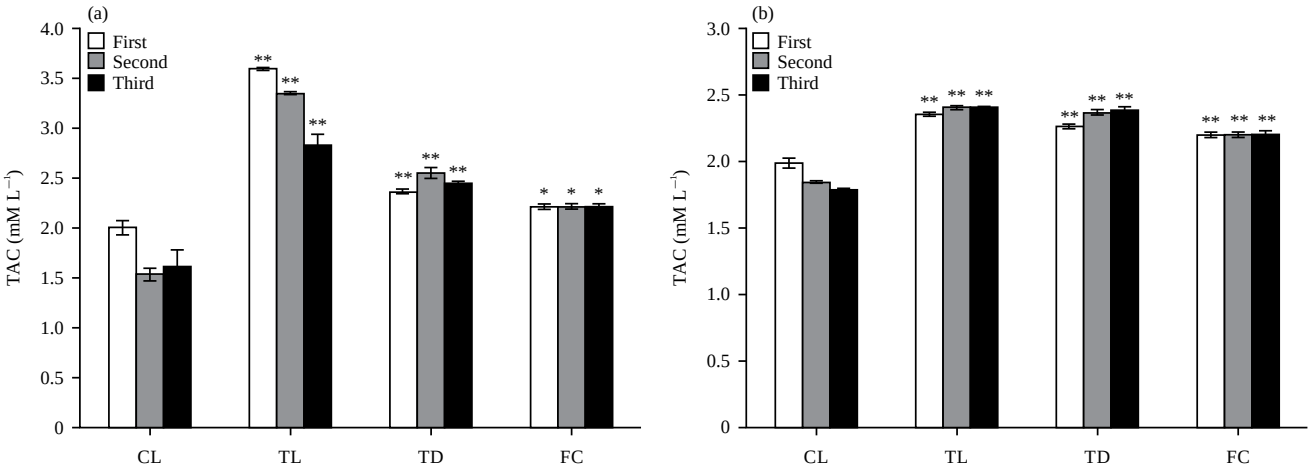

Fig. 1(a-b): Concentrations of TAC in adult *Apis mellifera* body homogenate after being treated with (a) 10^{-5} M L^{-1} copper chlorophyllin and (b) 3×10^{-3} magnesium chlorophyllin for different times

CL: Control light, TL: Treated light, TD: Treated dark and FC: Field collected groups, *,**Represent significant differences as compared to the CL group at p<0.01 and p<0.000, respectively

Table 1: Biochemical parameters of *Apis mellifera* homogenate after treated with 10^{-5} M L^{-1} copper and 3×10^{-3} M L^{-1} magnesium photosensitizer chlorophyllin for different times

	Photosensitizer	CL	TL	TD	FC
TP (µg µL^{-1})	Cu	0.99±0.2	1.8±0.04	1.29±0.18	1.08±0.2
		0.88±0.03	2.1±0.09	1.18±0.05	1.08±0.2
		1.22±0.01	1.88±0.1	1.46±0.2	1.11±0.2
	Mg	0.98±0.2	1.56±0.14	1.19±0.06	1.08±0.2
		1.01±0.4	1.76±0.03	1.22±0.03	1.08±0.2
		0.99±0.11	1.84±0.08	1.35±0.1	1.08±0.2
TAC (mM L^{-1})	Cu	2.01±0.12	3.59±0.01	2.37±0.03	2.22±0.05
		1.53±0.12	3.36±0.02	2.55±0.09	2.22±0.05
		1.60±0.3	2.82±0.2	2.47±0.00	2.22±0.05
	Mg	2.01±0.06	2.37±0.00	2.28±0.01	2.22±0.05
		1.85±0.00	2.42±0.01	2.39±0.00	2.22±0.05
		1.80±0.01	2.42±0.02	2.40±0.03	2.22±0.05
SOD (U μg^{-1} protein)	Cu	0.18±0.0218	0.43±0.0595	0.35±0.0409	0.21±0.064
		0.18±0.0174	0.32±0.0466	0.27±0.0599	0.23±0.064
		0.168±0.042	0.41±0.058	0.20±0.0460	0.21±0.064
	Mg	0.19±0.0485	0.28±0.057	0.25±0.0429	0.21±0.064
		0.18±0.050	0.27±0.057	0.26±0.0474	0.23±0.064
		0.19±0.0388	0.28±0.030	0.27±0.040	0.21±0.064
GST (U μg^{-1} protein)	Cu	1.51±0.07	2.40±0.25	2.01±0.14	2.00±0.25
		1.28±0.04	3.07±0.17	2.26±0.17	2.41±0.25
		1.31±0.14	2.56±0.06	1.98±0.27	2.45±0.25
	Mg	1.51±0.09	1.92±0.34	1.26±0.43	2.17±0.25
		1.47±0.68	2.32±0.01	1.65±0.20	2.22±0.25
		1.53±0.05	2.67±0.05	1.97±0.30	2.01±0.25

Honey bees treatment condition: CL: Control in light condition, TL: Treated in light condition, TD: Treated in dark condition and FC: Field collected bees, Measured biochemical parameters: TP: Total protein concentration in µg $\mu^{-}L^{1}$, TAC: Total antioxidant capacity in mM L^{-1}, SOD: Superoxide dismutase activity in U μg^{-1} protein and GST: Glutathione S-transferase activity in U μg^{-1} protein, (each measure represented as Mean±Standard error)

the TD group (Table 1, Fig. 3a and Supplemented Table 3). On the other hand, Mg-chlorophyllin fed bees exposed to light and dark conditions showed a gradual increase in GST activities with the highest value in the third day of feeding (TL 2.67±0.05 and TD 1.97±0.3 U μg^{-1} protein, respectively) Table 1 and Fig. 3b. Moreover, the GST activities measured from FC bees were higher than the TD group Table 1, Fig. 3b and Supplemented Table 3).

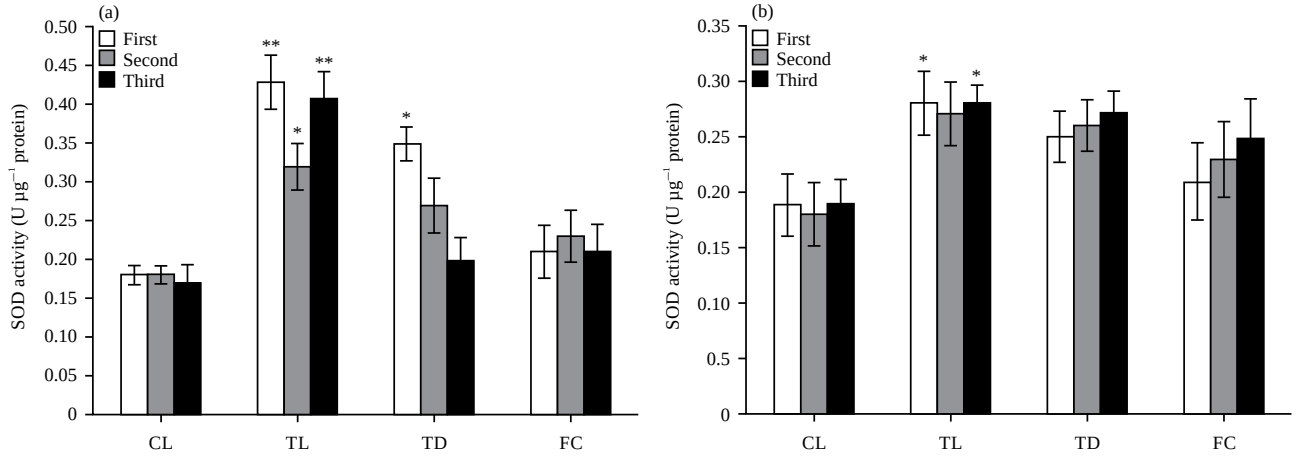

Fig. 2(a-b): Activities of SOD in adult *Apis mellifera* body homogenate after being treated with (a) 10^{-5} M L^{-1} copper chlorophyllin and (b) 3×10^{-3} magnesium chlorophyllin for different times

CL: Control light, TL: Treated light, TD: Treated dark and FC: Field-collected groups, *,**Represent significant differences as compared to the CL group at p<0.01 and p<0.000, respectively

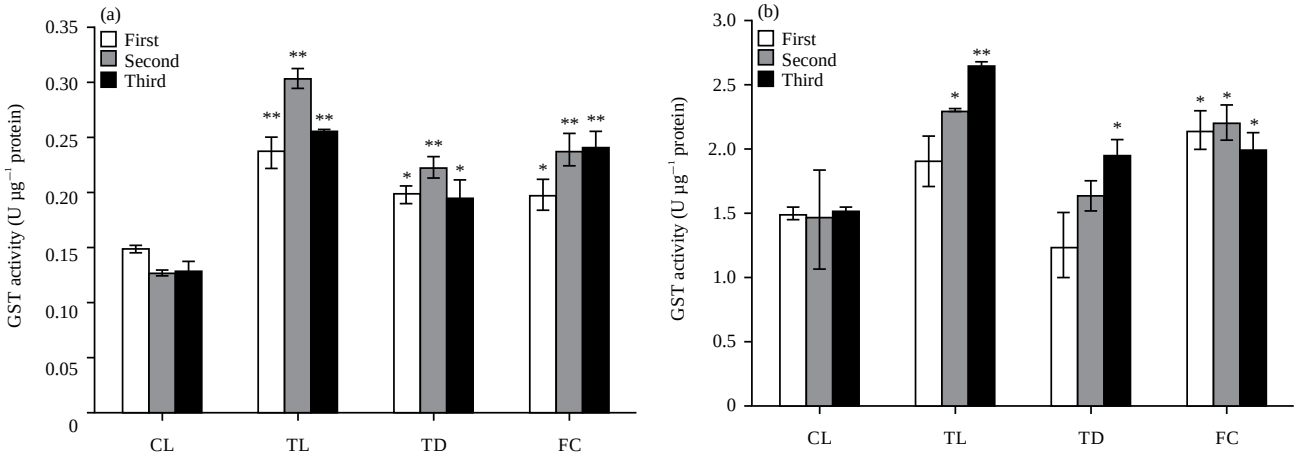

Fig. 3(a-b): Activities of GST in adult *Apis mellifera* body homogenate after being treated with (a) 10^{-5} M L^{-1} copper chlorophyllin and (b) 3×10^{-3} magnesium chlorophyllin for different times

CL: Control light, TL: Treated light, TD: Treated dark and FC: Field-collected groups, *,**Represent significant differences as compared to the CL group at p<0.01 and p<0.000, respectively

Table 2: Comet parameters in the adult *Apis mellifera* gut cells treated with 10^{-5} copper or 3×10^{-3} M L^{-1} magnesium chlorophyllin for three days

	Photosensitizer	CL	TL	TD
Tail length (µm)	Cu	1.24±0.251	3.26±0.424	2.56±0.424
	Mg	1.40±0.532	2.06±0.44	1.60±0.265
DNA damage in tail (%)	Cu	12.88±1.43	23.73±1.65	17.92±1.89
	Mg	12.86±2.44	16.34±1.43	13.76±1.49

Each measure represented as Mean±Standard error, Honey bees treatment condition: CL: Control exposed to light, TL: Treated exposed to light and TD: Treated kept in dark

Genotoxic effect of chlorophyllin derivatives on honey bee: Gut cells of honey bees fed on Cu or Mg-chlorophyllin for three days were used for quantitative analysis of DNA damage by comet assay and expressed as tail length (TL) (µm), DNA tail% and the tail moment. The midgut cell nuclei with tail-like extension was an indication for DNA damage while the intact ones were almost rounded (Fig. 4a). The obtained TL (µm) in Cu-chlorophyllin fed bees in light (TL: 3.26±0.424 µm) and

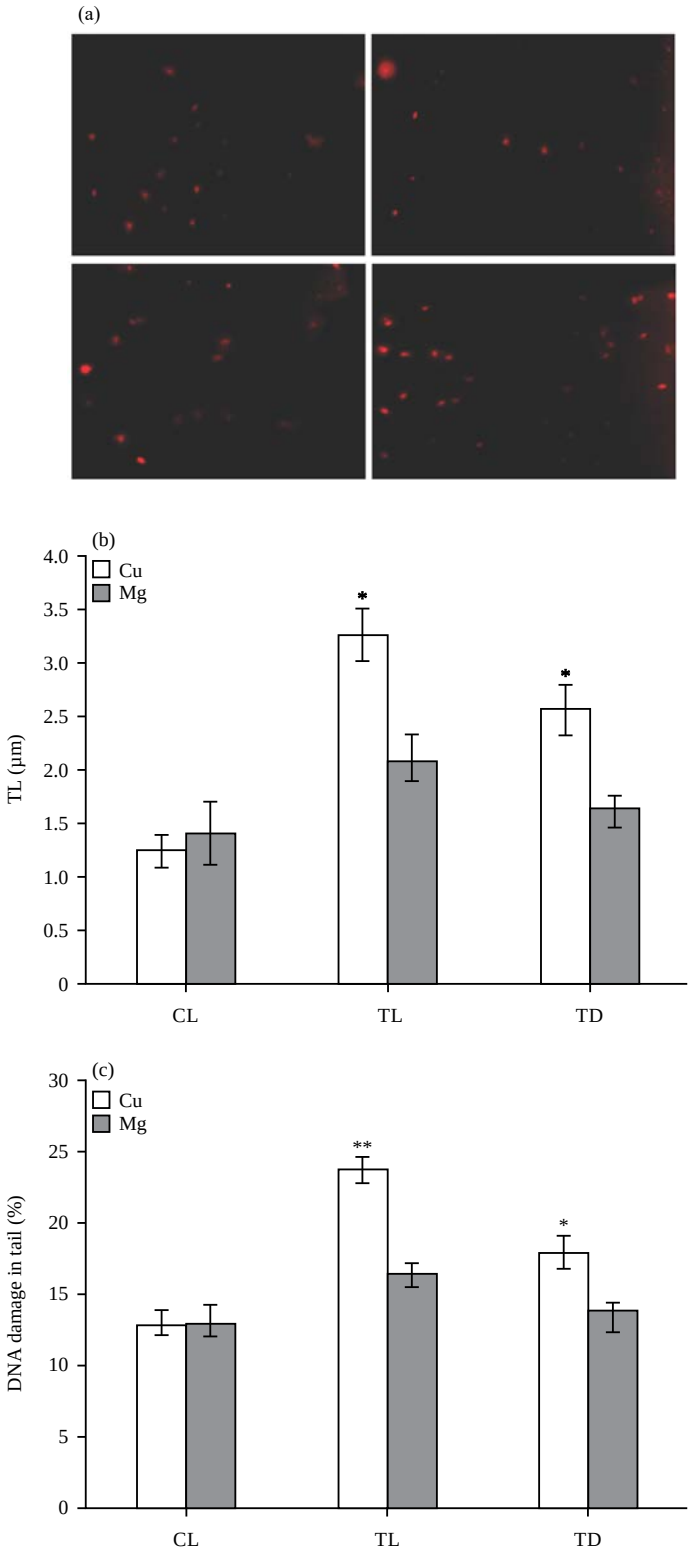

Fig. 4(a-c): Different cell damage stages in the comet assay in *Apis mellifera* worker after treatment with (a) Chlorophyllin derivatives and exposed to sunlight, (b and c) Comet parameters in the adult *Apis mellifera* gut cells after 3 days fed with 10^{-5} M L^{-1} copper or 3×10^{-3} magnesium chlorophyllin

CL: Control light, TL: Treated light and TD: Treated dark groups, *,**Represent significant differences as compared to the CL group at $p<0.01$ and $p<0.000$, respectively

dark condition (TD: 2.56±0.424 µm) showed a significant increase compared with the control group (Fig. 4b). Also, the DNA damage measured as (DNA% in Tail) presented a clear and significant indication of genotoxicity of chlorophyllin derivatives. The percentage of DNA damage was 23.73±1.65 and 16.34±1.43 in the bees' gut cells fed on Cu-chlorophyllin and Mg-chlorophyllin which were exposed to sunlight (TL), Table 2 and Fig. 4c. The minimum percent of DNA in the tail was measured in sugar-fed bees exposed to light, CL (12.86±2.44). Under the dark condition, the Cu-chlorophyllin fed bees showed DNA damage (17.92±1.89) more than the Mg-chlorophyllin fed bee (13.67±1.49) under Table 2 and Fig. 4c.

DISCUSSION

All aerobic organisms possess antioxidant systems, which function to prevent oxidative damage of ROS resulting from internal metabolism or due to exposure to insecticides including organophosphates, carbamates and pyrethroids[7,11]. The present study was aimed to assess the oxidative stress and genotoxicity effects in *A. mellifera* under the influence of Cu or Mg-chlorophyllin. The TAC of bee body homogenate was significantly increased upon feeding on 10^{-5} M L^{-1} Cu-chlorophyllin for three days under light and dark conditions compared to the control and field-collected groups (Table 1 and Fig. 1a). In the contrast, the Mg-chlorophyllin fed bees showed a little increase in TAC compared to the control group and it was almost the same TAC measured in the TD and FC groups (Table1 and Fig. 1b). Similarly, Nicewicz *et al.*[35] observed a higher TAC (enzymatic and non-enzymatic antioxidant) in the fat body of rural bees. The increased TAC met the elevation of oxidative stress upon feeding on chlorophyllin and thus the antioxidant capabilities[36]. The SOD enzyme is one of the antioxidant protection systems against Reactive Oxygen Species (ROS)[37,38], which increased due to exposure to many agrochemicals (such as pesticides, herbicides, extra) and heavy metals[39-41]. SOD activity was significantly increased in Cu-chlorophyllin fed bees and its highest activities were on the first day of feeding both in TL (0.32±0.04663 U μg^{-1} protein) and TD (0.35±0.0409 U μg^{-1} protein) groups (Table 1 and Fig. 2a). These results were inconsistent with Bernardes *et al.*[8], who have observed a significant increase in CAT and SOD activities of the stingless bee *Partamona helleri* exposed to $CuSO_4$ (LC_{50} = 142.95 µg mL^{-1}). Also, CAT and SOD increased in the foragers of *P. helleri* that were exposed to the insecticide fipronil[41], indicating that different agrochemicals can induce oxidative stress on these bees.

GST is a multifunctional enzyme that works to neutralize toxic compounds by conjugating them to glutathione for removal from cells[42-45]. So, GST activity in Cu-chlorophyllin fed bees referred to the metabolic actions against both the chlorophyllin and copper elements parts. These results agreed with Nikolić *et al.*[46], who recorded that an increase in Cu and Cd concentration added to sucrose fed to bees caused an increase of three GST activities and expression level Furthermore, the measured GST activity in the field-collected bees was higher than that in the Mg-chlorophyllin fed bees which might reflect the harmful substances such as pesticides and xenobiotics the bees were exposed in the field. These results agreed with Orčić *et al.*[47], who indicated that SOD and GST activity increased in summer workers compared to winter worker bees.

Animal cells exposed to ROS that are released due photopesticide activity have shown a different level of cellular compartment damage[48,49]. The single-cell electrophoresis, comet assay is used for studying environmental pollution risk at the level of genetic materials[50,51]. There is no information on the effects of chronic exposure to photo-pesticides on honey bees. This is, to our knowledge, the first study that utilized comet assay to reveal genotoxicity of photo-pesticides on worker forage bees. The DNA damage measured as percent of Tail DNA was significantly higher in gut cells of forage bee fed on Cu-chlorophyllin compared to Mg-chlorophyllin. This damage was also highly and significantly correlated with the accumulation of chlorophyllin derivatives in the bee body and can be reduced through the active antioxidant system (high efficiency of release dynamic) of honey bees which decreased the photooxidation stress of Cu-chlorophyllin and Mg-chlorophyllin by reducing their accumulated concentration in two days maximum. This result may be due to Cu metal which produces OH that interacts with cellular redox leading to ROS formation and oxidative DNA damage[52,53]. Also, the DNA repair system is reduced by the free metal[51-55]. This study presented an assessment of domesticated honey bee response to photo-pesticide exposures which could be critical to determine non-target pesticide impacts on such economically important insects.

CONCLUSION

The finding of this research recommended chlorophyllin derivatives field application to control pest insects such as mosquitoes and lepidopteran larvae without affecting the beneficial insect such as a honey bee. We are preparing to study the effect of the same chlorophyllin derivatives on other carnivores and parasitoids insects. The overall aim is to recover the natural balance of organisms destroyed by human activities.

SIGNIFICANCE STATEMENT

The applied objective of this study is to use a safe and quick method to assess the expected toxicity of chlorophyll derivatives, photosynthetically, before recommending its use in the open field on large scale. This study will help researchers to use chlorophyllin derivatives in the field control of pests within the safe concentration for honey bees, as there is not a sufficient number of studies on the environmental toxicity of these compounds on beneficial organisms. Thus a new theory about biosafety can be followed.

REFERENCES

1. Allsopp, M.H., W.J. de Lange and R. Veldtman, 2008. Valuing insect pollination services with cost of replacement. PLOS ONE, Vol. 3. 10.1371/journal.pone.0003128.
2. Leonhardt, S.D., N. Gallai, L.A. Garibaldi, M. Kuhlmann and A.M. Klein, 2013. Economic gain, stability of pollination and bee diversity decrease from Southern to Northern Europe. Basic Appl. Ecol., 14: 461-471.
3. Alaux, C., J.L. Brunet, C. Dussaubat, F. Mondet and S. Tchamitchan *et al*., 2010. Interactions between *Nosema* microspores and aneonicotinoid weaken honey bees (*Apis mellifera*). Environ. Microbiol., 12: 774-782.
4. Cornman, R.S., D.R. Tarpy, Y. Chen, L. Jeffreys and D. Lopez *et al*., 2012. Pathogen webs in collapsing honey bee colonies. PLOS ONE, Vol. 7. 10.1371/journal.pone.0043562.
5. Rothman, J.A., L. Leger, J.S. Kirkwood and Q.S. McFrederick, 2019. Cadmium and selenate exposure affects the honey bee microbiome and metabolome and bee-associated bacteria show potential for bioaccumulation. Appl. Environ. Microbiol., Vol. 85. 10.1128/aem.01411-19.
6. Li, X., W. Ma, J. Shen, D. Long and Y. Feng *et al*., 2019. Tolerance and response of two honeybee species *Apis cerana* and *Apis mellifera* to high temperature and relative humidity. PLOS ONE, Vol. 14. 10.1371/journal.pone.0217921.
7. Gizaw, G., Y. Kim, K. Moon, J.B. Choi, Y.H. Kim and J.K. Park, 2020. Effect of environmental heavy metals on the expression of detoxification-related genes in honey bee *Apis mellifera*. Apidologie, 51: 664-674.
8. Bernardes, R.C., K.M. Fernandes, D.S.S. Bastos, A.F.P.A. Freire and M.P. Lopes *et al*., 2021. Impact of copper sulfate on survival, behavior, midgut morphology and antioxidant activity of *Partamona helleri* (Apidae: Meliponini). Environ. Sci. Pollut. Res., 29: 6294-6305.
9. Henry, M., M. Beguin, F. Requier, O. Rollin and J.F. Odoux *et al*., 2012. A common pesticide decreases foraging success and survival in honey bees. Science, 336: 348-350.
10. Molina, M.J. and L.T. Molina, 2004. Megacities and atmospheric pollution. J. Air Waste Manage. Assoc., 54: 644-680.
11. Noi, A.D., S. Casini, T. Campani, G. Cai and I. Caliani, 18. Review on sublethal effects of environmental contaminants in honey bees (*Apis mellifera*), knowledge gaps and future perspectives. Int. J. Environ. Res. Public Health, Vol. 18. 10.3390/ijerph18041863.
12. Caliani, I., T. Campani, B. Conti, F. Cosci and S. Bedini *et al*., 2021. Multi-biomarker approach and IBR index to evaluate the effects of different contaminants on the ecotoxicological status of *Apis mellifera*. Ecotoxicol. Environ. Saf., 10.1016/j.ecoenv.2020.111486.
13. Weirich, G.F., A.M. Collins and V.P. Williams, 2002. Antioxidant enzymes in the honey bee, *Apis mellifera*. Apidologie, 33: 3-14.
14. Lemaitre, B. and J. Hoffmann, 2007. The host defense of *Drosophila melanogaster*. Ann. Rev. Immunol., 25: 697-743.
15. ALNaggar, Y., A. Vogt, G. Codling, E. Naiem and M. Mona, 2015. Exposure of honeybees (*Apis mellifera*) in Saskatchewan, Canada to organophosphorus insecticides. Apidologie, 46: 667-678.
16. Qi, S., X. Niu, D. hui Wang, C. Wang and L. Zhu *et al*., 2020. Flumethrin at sublethal concentrations induces stresses in adult honey bees (*Apis mellifera* L.). Sci. Total Environ., Vol. 700. 10.1016/j.scitotenv.2019.134500.
17. Almasri, H., D.A. Tavares, M. Pioz, D. Sené and S. Tchamitchian *et al*., 2020. Mixtures of an insecticide, a fungicide and a herbicide induce high toxicities and systemic physiological disturbances in winter *Apis mellifera* honey bees. Ecotoxicol. Environ. Saf., 10.1016/j.ecoenv.2020.111013.
18. Paillous, N. and S. Fery-Forgues, 1994. Interest of photochemical methods for induction of lipid peroxidation. Biochimie, 76: 355-368.
19. Davies, K.J., 2000. Oxidative stress, antioxidant defenses and damage removal, repair and replacement systems. IUBMB Life, 50: 279-289.
20. Kramer, B.H., V. Nehring, A. Buttstedt, J. Heinze and J. Korb *et al*., 2021. Oxidative stress and senescence in social insects: A significant but inconsistent link? Philos. Trans. Royal Soc. B: Bio. Sci., Vol. 376. 10.1098/rstb.2019.0732.
21. Pérez-Gálvez, A., I. Viera and M. Roca, 2020. Carotenoids and chlorophylls as antioxidants. antioxidants, Vol. 9. 10.3390/antiox9060505.
22. Abdel-Kader, M.H. and T.A. El-Tayeb, 2012. Field implementation using chlorophyll derivatives with sunlight for malaria, filaria and dengue fever vectors control in infested Africa swamps. Malaria J., Vol. 11. 10.1186/1475-2875-11-s1-p42.
23. Abdel-Kader, M.H. and T.A. Eltayeb, 2014. Photodynamic Control of Malaria Vector, Noxious Insects and Parasites. In: Photodynamic Therapy. Abdel-Kader, M.H. (Ed.). Springer Berlin Heidelberg, Germany.

24. Afify, A., N.A. Ghezawy, M.M. Ali and T.A. El-Tayeb, 2019. Effects of copper chlorophylline and magnesium chlorophylline on the humeral immune response, ultrastructural changes of midgut and DNA damage to *Culex pipiens* larvae. Afr. Entomol., 27: 146-158.
25. Nassar, S.M., A.M. Elgendy and T.A. El-Tayeb, 2021. Assessement of chlorophyll phototoxicity on honey bee, *Apis mellifera* (Hymenoptera: Apidae). J. Egypt. Soc. Parasitol., 51: 63-72.
26. Decourtye, A., J. Devillers, E. Genecque, K. Le Menach, H. Budzinski, S. Cluzeau and M.H. Pham-Delègue , 2005. Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. Arch. Environ. Contam. Toxicol., 48: 242-250.
27. de Castro, M.B.A., L.C. Martinez, J.F.S. Cossolin, R.S. Serra and J.E. Serrão, 2020. Cytotoxic effects on the midgut, hypopharyngeal, glands and brain of *Apis mellifera* honey bee workers exposed to chronic concentrations of lambda-cyhalothrin. Chemosphere, Vol. 248. 10.1016/j.chemosphere.2020.126075.
28. Abou-Shaara, H.F., 2014. The foraging behaviour of honey bees, *Apis mellifera*: A review. Vet. Med., 59: 1-10.
29. Badawy, M.E.I., H.M. Nasr and E.I. Rabea, 2014. Toxicity and biochemical changes in the honey bee *Apis mellifera* exposed to four insecticides under laboratory conditions. Apidologie, 46: 177-193.
30. Koracevic, D., G. Koracevic, V. Djordjevic, S. Andrejevic and V. Cosic, 2001. Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol., 54: 356-361.
31. Nishikimi, M., N.A. Rao and K. Yog, 1972. Colorimetric determination of superoxide dismutase activity. Biochem. Biophys. Res. Commun., 46: 849-851.
32. Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.
33. Gornall, A.G., C.J. Bardawill and M.M. David, 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177: 751-766.
34. Kumaravel, T.S., B. Vilhar, S.P. Faux and A.N. Jha, 2007. Comet assay measurements: A perspective. Cell Biol. Toxicol., 25: 53-64.
35. Nicewicz, Ł., A.W. Nicewicz, A. Kafel and M. Nakonieczny, 2020. Set of stress biomarkers as a practical tool in the assessment of multistress effect using honeybees from urban and rural areas as a model organism: A pilot study. Environ. Sci. Pollut. Res., 28: 9084-9096.
36. Słowińska, M., J. Nynca, J. Wilde, B. Bąk, M. Siuda and A. Ciereszko, 2015. Total antioxidant capacity of honeybee haemolymph in relation to age and exposure to pesticide and comparison to antioxidant capacity of seminal plasma. Apidologie, 47: 227-236.
37. Birben, E., U.M. Sahiner, C. Sackesen, S. Erzurum and O. Kalayci, 2012. Oxidative stress and antioxidant defense. World Allergy Organiz. J., 5: 9-19.
38. Hsieh, Y.S. and C.Y. Hsu, 2013. Oxidative stress and anti-oxidant enzyme activities in the trophocytes and fat cells of queen honeybees (*Apis mellifera*). Rejuvenation Res., 16: 295-303.
39. Alaraby, M., A. Hernández and R. Marcos, 2016. New insights in the acute toxic/genotoxic effects of CuO nanoparticles in the *in vivo Drosophila* model. Nanotoxicology, 10: 749-760.
40. Yang, F., R. Pei, Z. Zhang, J. Liao and W. Yu *et al*., 2019. Copper induces oxidative stress and apoptosis through mitochondria-mediated pathway in chicken hepatocytes. Toxicol. *In vitro*, 54: 310-316.
41. Farder-Gomes, C.F., K.M. Fernandes, R.C. Bernardes, D.S.S. Bastos, G.F. Martins and J.E. Serrão, 2021. Acute exposure to fipronil induces oxidative stress, apoptosis and impairs epithelial homeostasis in the midgut of the stingless bee *Partamona helleri* friese (Hymenoptera: Apidae). Sci. Total Environ., Vol. 774. 10.1016/j.scitotenv.2021.145679.
42. Sanchez-Bayo, F. and K. Goka, 2014. Pesticide residues and bees-A risk assessment. PLOS ONE, Vol. 9. 10.1371/journal.pone.0094482.
43. Claudianos, C., H. Ranson, R.M. Johnson, S. Biswas and M.A. Schuler *et al*., 2006. A deficit of detoxification enzymes: Pesticide sensitivity and environmental response in the honeybee. Insect Mol. Biol., 15: 615-636.
44. Badiou-Bénéteau, A., S.M. Carvalho, J.L. Brunet, G.A. Carvalho, A. Buleté, B. Giroud and L.P. Belzunces, 2012. Development of biomarkers of exposure to xenobiotics in the honey bee *Apis mellifera*: Application to the systemic insecticide thiamethoxam. Ecotoxicol. Environ. Saf., 82: 22-31.
45. Carvalho, S.M., L.P. Belzunces, G.A. Carvalho, J.L. Brunet and A. Badiou-Beneteau, 2013. Enzymatic biomarkers as tools to assess environmental quality: A case study of exposure of the honeybee *Apis mellifera* to insecticides. Environ. Toxicol. Chem., 32: 2117-2124.
46. Nikolić, T.V., D. Kojić, S. Orčić, E.L. Vukašinović, D.P. Blagojević and J. Purać, 2019. Laboratory bioassays on the response of honey bee (*Apis mellifera* L.) glutathione S-transferase and acetylcholinesterase to the oral exposure to copper, cadmium and lead. Environ. Sci. Pollut. Res., 26: 6890-6897.
47. Orčić, S., T. Nikolić, J. Purać, B. Šikoparija and D.P. Blagojević *et al*., 2017. Seasonal variation in the activity of selected antioxidant enzymes and malondialdehyde level in worker honey bees. Entomol. Exp. Appl. 165: 120-128.
48. de Lapuente, J., J. Lourenço, S.A. Mendo, M. Borràs, M.G. Martins, P.M. Costa and M. Pacheco, 2015. The comet assay and its applications in the field of ecotoxicology: A mature tool that continues to expand its perspectives. Front. Genet., Vol. 6. 10.3389/fgene.2015.00180.
49. Gajski, G., B. Žegura, C. Ladeira, B. Pourrut and C.D. Bo' *et al*., 2019. The comet assay in animal models: From bugs to whales-(part 1 invertebrates). Mutat. Res./Rev. Mutat. Res., 779: 82-113.
50. Augustyniak, M., M. Gladysz and M. Dziewięcka, 2016. The comet assay in insects-status, prospects and benefits for science. Mutat. Res./Rev. Mutat. Res., 767: 67-76.

51. Meland, S., T. Gomes, K. Petersen, J. Håll, E. Lund, A. Kringstad and M. Grung, 2019. Road related pollutants induced DNA damage in dragonfly nymphs (Odonata, Anisoptera) living in highway sedimentation ponds. Sci. Rep., Vol. 9. 10.1038/s41598-019-52207-4.
52. Matzenbacher, C.A., A.L.H. Garcia, M.S. dos Santos, C.C. Nicolau and S. Premoli *et al*., 2017. Dna damage induced by coal dust, fly and bottom ash from coal combustion evaluated using the micronucleus test and comet assay *in vitro*. J. Hazard. Mater., 324: 781-788.
53. Wu, X., S.J. Cobbina, G. Mao, H. Xu, Z. Zhang and L. Yang, 2016. A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. Environ. Sci. Pollut. Res., 23: 8244-8259.
54. Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol., 101: 13-30.
55. Lushchak, V.I., 2011. Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., 153: 175-190.

Supplemented Table 1: Effect of experimental conditions (EC) on TP and TAC in adult *Apis mellifera* body homogenate after treated with 10^{-5} M L^{-1} copper and 3×10^{-3} M L^{-1} magnesium chlorophyllin for different times

	Photosensitizer	Time (days)	Factor	Sum of squares	df	Mean square	$F_{calculated}$	p-value
TP	Cu	First	EC	1.309	3	0.436	15.305	0.001
			Error	0.228	8	0.028		
			Total	1.537	11			
		Second	EC	2.636	3	0.879	68.256	0.000
			Error	0.103	8	0.013		
			Total	2.739	11			
		Third	EC	1.048	3	0.349	18.456	0.001
			Error	0.151	8	0.019		
			Total	1.199	11			
	Mg	First	EC	0.577	3	0.192	5.886	0.02
			Error	0.262	8	0.033		
			Total	0.839	11			
		Second	EC	1.039	3	0.346	31.915	0.000
			Error	0.087	8	0.011		
			Total	1.126	11			
		Third	EC	1.313	3	0.438	25.559	0.000
			Error	0.137	8	0.017		
			Total	1.45	11			
TAC	Cu	First	EC	4.543	3	1.514	338.43	0.000
			Error	0.036	8	0.004		
			Total	4.579	11			
		Second	EC	5.198	3	1.733	272.835	0.000
			Error	0.051	8	0.006		
			Total	5.248	11			
		Third	EC	2.359	3	0.786	23.665	0.000
			Error	0.266	8	0.033		
			Total	2.625	11			
	Mg	First	EC	0.211	3	0.07	42.545	0.000
			Error	0.013	8	0.002		
			Total	0.224	11			
		Second	EC	0.599	3	0.2	201.465	0.000
			Error	0.008	8	0.001		
			Total	0.607	11			
		Third	EC	0.745	3	0.248	254.769	0.000
			Error	0.008	8	0.001		
			Total	0.753	11			

Measured biochemical parameters: TP: Total protein concentration in µg μL^{-1} and TAC: Total antioxidant capacity in mM L^{-1}. $p>0.05$: Insignificant effect, $p<0.000$, $p<0.01$ and $p<0.05$: Significant effect

Supplemented Table 2: Activities of GST and SOD in adult *Apis mellifera* body homogenate after treated with 10^{-5} M L^{-1} copper and 3×10^{-3} magnesium photosensitizer chlorophyllin for different times

	Photosensitizer	Time (days)	Factors	Sum of squares	df	Mean square	$F_{calculated}$	p-value
GST	Cu	First	EC	1.196	3	0.399	10.665	0.004
			Error	0.299	8	0.037		
			Total	1.495	11			
		Second	EC	4.917	3	1.639	53.779	0.000
			Error	0.244	8	0.030		
			Total	5.161	11			

Supplemented Table 2: Continue

	Photosensitizer	Time (days)	Factors	Sum of squares	df	Mean square	$F_{calculated}$	p-value
		Third	EC	2.910	3	0.970	24.467	0.000
			Error	0.317	8	0.040		
			Total	3.228	11			
	Mg	First	EC	1.494	3	0.498	5.369	0.026
			Error	0.742	8	0.093		
			Total	2.236	11			
		Second	EC	1.576	3	0.525	3.719	0.061
			Error	1.130	8	0.141		
			Total	2.706	11			
		Third	EC	1.988	3	0.663	24.659	0.000
			Error	0.215	8	0.027		
			Total	2.203	11			
SOD	Cu	First	EC	0.125	3	0.042	18.12	0.001
			Error	0.018	8	0.002		
			Total	0.143	11			
		Second	EC	0.032	3	0.011	4.198	0.046
			Error	0.02	8	0.003		
			Total	0.052	11			
		Third	EC	0.108	3	0.036	12.77	0.002
			Error	0.023	8	0.003		
			Total	0.131	11			
	Mg	First	EC	0.015	3	0.005	1.912	0.206
			Error	0.02	8	0.003		
			Total	0.035	11			
		Second	EC	0.015	3	0.005	1.922	0.205
			Error	0.02	8	0.003		
			Total	0.035	11			
		Third	EC	0.015	3	0.005	2.532	0.130
			Error	0.015	8	0.002		
			Total	0.03	11			

Measured biochemical parameters: SOD: Superoxide dismutase activity in U μg^{-1} protein and GST: Glutathione S-transferase activity in U μg^{-1} protein. p>0.05: Insignificant effect, p<0.000, p<0.01 and p<0.05: Significant effect

Supplemented Table 3: Comet parameter in the adult gut cell of *Apis mellifera* after fed with 10^{-5} M L^{-1} copper and 3×10^{-3} M L^{-1} magnesium chlorophyllin for three days

	Photosensitizer	Factor	Sum of squares	df	Mean square	$F_{calculated}$	p-value
TL	Cu	EC	6.313	2	3.156	22.801	0.002
		Error	0.831	6	0.138		
		Total	7.143	8			
	Mg	EC	0.685	2	0.343	1.888	0.231
		Error	1.089	6	0.182		
		Total	1.775	8			
TM	Cu	EC	0.072	2	0.036	1.395	0.318
		Error	0.155	6	0.026		
		Total	0.228	8			
	Mg	EC	0.764	2	0.382	53.042	0.000
		Error	0.043	6	0.007		
		Total	0.807	8			
DNA (%)	Cu	EC	176.880	2	88.44	31.815	0.001
		Error	16.679	6	2.78		
		Total	193.559	8			
	Mg	EC	19.725	2	9.862	2.895	0.132
		Error	20.437	6	3.406		
		Total	40.162	8			

CL: Control light, TL: Treated light and TD: Treated, p>0.05: Insignificant effect, p<0.000, p<0.01 and p<0.05: Significant effect