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Research Article

Impact of Silicon Dioxide Nanoparticles on Nutritional Composition of Edible Insect: *Galleria mellonella* (Lepidoptera: Pyralidae) Larvae

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ABSTRACT

The present study investigates the impact of SiO_2 NPs (Silicon Dioxide Nanoparticles) (500–60000 ppm) on key chemical parameters including protein, lipid, carbohydrate, moisture, ash, and fatty acid composition in *Galleria mellonella* (Lepidoptera: Pyralidae) larvae, with a view to identifying potential implications for sustainable food systems. It was determined that the protein and carbohydrate contents of larvae fed with high doses (>5000 ppm) of SiO_2 NPs were significantly reduced in comparison to the control group. Moreover, an increment in the dose of SiO_2 NPs resulted in a decrease in the fat content of the larvae. It was found that larvae exposed to 500 and 30000 ppm SiO_2 NPs exhibited a reduction in moisture content. Furthermore, the ash content of all larvae treated with SiO_2 NPs exhibited a significant increase. Finally, an increment in the dose of SiO_2 NPs in the larvae was found to be an increase in the level of palmitic acid and a decrease in the level of oleic acid. These findings demonstrate the importance of evaluating the risks associated with nanoparticle exposure in edible insect-based food products with a view to ensuring food safety and sustainability.

1. Introduction

The global food system is facing two significant challenges: The necessity of feeding an expanding population and the responsibility to minimize its environmental impact [1]. The production of conventional protein sources, such as livestock, is characterized by a high environmental impact such as gas emissions, excessive freshwater use, and large-scale deforestation and is a significant contributor to environmental degradation [2]. It is therefore evident that the examination of sustainable and alternative protein sources has become a crucial field of research and development, with the objective of ensuring global food security [3].

Edible insects, which are an important source of protein, lipids, and some essential components, represent a promising solution to this global challenge [3, 4]. The high protein content and adaptability of The Greater Wax Moth (*Galleria mellonella*) (Lepidoptera: Pyralidae) larvae make them a particularly suitable model for the study of nanoparticle interactions and their nutritional implications. The larvae of the *G. mellonella* represent a particularly noteworthy species within the diverse range of edible insects (it is not consumed directly by humans, generally it is used in zoos for insectivorous creatures). They have attracted considerable interest due to the ease with which they can be cultivated, their high nutritional value, and their adaptability.

The larvae depending on species and stage have been found to contain high levels of lipids (2–62%), and especially oleic and linoleic acids, which are considered essential for human health

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[5-7]. Furthermore, it is notable that the protein content exceeds averagely 30%. It has been reported that albumins (>45%) and glutelins (>35%) are the dominant proteins in the *G. mellonella* protein composition, with prolamins and globulins also reported [8]. The protein content of *G. mellonella* is considerably higher than that of conventional meat products and several plant-based protein sources [9]. Additionally, it has been indicated that insect proteins are comparatively more digestible than plant proteins [10]. Therefore, their nutritional profile, which includes protein, lipid, and carbohydrate reserves, makes them an attractive dietary substitute to meet the increasing global food demands [11, 12].

Although the potential of *G. mellonella* as a sustainable protein source is evident, it is important to note that the nutritional composition and quality of these larvae can be influenced by environmental exposures [13]. For example, their composition can change with exposure to various stressors such as temperature, humidity, oxygen including external chemicals or nanoparticles. Furthermore, the nutritional composition may also be changed to interactions with components commonly found in nature or food additives utilized in food applications. Silicon dioxide (SiO_2) is a notable component due to its extensive utilization and potential interactions with biological systems. SiO_2 is a metal oxide in its amorphous form and was approved as a food additive by European Food Safety Authority (EFSA) and Food and Drug Administration (FDA) [14, 15].

It has been utilized for several decades as an anti-caking, stabilizer and adsorbent in some foods and dietary foods [16, 17]. The SiO_2 is a particulate material, and nano-sized particles (1–100 nm) are probably formed during its manufacture. It has been demonstrated in previous studies that SiO_2 NPs have a damaging effect on a range of organisms, resulting in reduced survival rates, diminished cellular viability, and modifications to lipid metabolism [18-20]. Furthermore, our previous research indicated a notable reduction in hemocyte counts and viability in *G. mellonella* larvae following exposure to elevated doses of SiO_2 NPs [21]. Nevertheless, the extent to which such exposures affect the nutritional quality of *G. mellonella* larvae remains largely uninvestigated. Therefore, this study aims to address the lack of research in this area by investigating the effects of varying concentrations of SiO_2 NPs on the chemical and biochemical parameters of *G. mellonella* larvae. In particular, the impact of the nanoparticles on the nutritional value of the larvae was assessed by changes in protein, lipid, carbohydrate, moisture, ash, and fatty acid composition. By connecting the effects of nanoparticle exposure to nutritional outcomes, this research contributes to the expanding field of knowledge regarding edible insects and their part in sustainable food systems.

2. General Methods

2.1. Insect

The larvae were reared in conditions of 25±5°C, 60±5% relative humidity and a photoperiod of 12:12 (light: dark). The rearing conditions, including temperature, humidity and ambient light, were rigorously monitored and maintained throughout the experimental period. Adult insects and newly hatched larvae were raised in glass jars and honeycomb was used for their nutrition. A spherical nanopowder of SiO_2 NPs with a diameter of 22 nm was employed in all experimental treatments (Nanokar, İstanbul, Türkiye).

2.2. Characterization of SiO_2 NPs

Scanning electron microscopy (SEM) and X-ray diffraction (XRD) were employed to confirm the morphological and structural properties of the SiO_2 NPs, thereby ensuring consistency and accuracy in the experimental treatments. Results of spherical and 22 nm-sized hydrophilic amorphous SiO_2 NPs were given in detail in a study that we have previously performed [21]. The XRD results of the SiO_2 NPs revealed the presence of an amorphous peak with an equivalent Bragg angle of $2\theta=22.16$. [21, 22].

2.3. Experimental diets

A series of multiple-dose experiments at elevated doses was conducted to ascertain the LD_{50} (lethal dose) of SiO_2 NPs. However, the doses of 500,

5000, 30000, and 60000 ppm of SiO_2 NPs were identified as the experimental doses for the study, as the mortality rates observed in larvae exposed to doses below 60000 ppm were within the range of 50-90% [23]. In the study, doses of 500, 5000, 30000, and 60000 ppm SiO_2 NPs were added to the insect diet mixture recommended by Bronskill [24]. The SiO_2 NPs were sonicated in a bath sonicator for 5 min before being transferred to the experimental diets. Only pure water was added to diets of the control larvae. Forty second instar larvae were transferred to the insect feeding diets. From these larvae, 14-16 days old last instar larvae were selected for the treatments. For each experimental group, 12 larvae, with 3 repetitions and 4 larvae in each repetition were selected so that their total weight would be equivalent to 2g [25, 26]. The moisture (mg), protein (%), lipid (%), carbohydrates (%), ash content (%) and fatty acid composition of the larvae was determined.

2.4. Moisture content analysis

The samples were subjected to a drying process at 65°C for approximately 8 h until a constant weight was attained. Subsequently, the moisture content of the larvae was calculated by subtracting the dry weight from the fresh weight [27].

2.5. Ash content analysis

The samples were weighted in porcelain crucible and heated at 550°C for 12 h. Samples reached a constant weight and light grey color after the heating. After the samples were cooled to room temperature, ash content was calculated from the weight difference [28].

2.6. Protein content analysis

The protein content of samples was determined by the Kejldahl method [29]. The samples were weighted into the digestion tubes and the catalyst (K_2SO_4 and $CuSO_4$) and H_2SO_4 were added to the tubes. The samples were digested until the mixture reached a green color. After digestion, samples were distillated with Na_2SO_4 solution into the H_3BO_3 solution. Finally, distillated samples were titrated by HCl and the nitrogen content was calculated. In order to determine the total protein amount, the nitrogen content determined was multiplied by a coefficient of 5.6 [30].

2.7. Fat content analysis

The fat content of the larvae was determined by the Soxhlet extraction method [28]. The sample was weighted into Soxhlet apparatus and petroleum ether was added as solvent. The sample was extracted for a total of 6 h. The fat content of the sample was calculated based on the weight difference before and after extraction.

2.8. Carbohydrates content analysis

The carbohydrate content of the larvae was determined by the anthrone method [31]. Dried samples were stirring with distilled water at 25°C for 1 h and then centrifuged to obtain extraction. Extracts were mixed with anthrone reagent and mixed for 1 min. Then, mixture were heated at 100°C for 30 min. The absorbance of samples were determined at 620 nm by UV-VIS spectrophotometer after the samples cooled to room temperature. The results were expressed as percentage of dry sample mass.

2.9. Fatty acid composition analysis

The cuticular free fatty acids were extracted by method described by [26]. The samples were extracted for a period of 5 min in 20 ml of petroleum ether, followed by a further 5 min in 20 ml of dichloromethane. The methylation procedure of lipids and GC condition was carried out in accordance with the methodology described by Ozer and Kilic [32]. Extracted lipid from the larvae was methylated with CH_3ONa solution in methanol and BF_3 solution in methanol and analyzed by Agilent 7820A gas chromatography (Agilent Technologies, USA). The identification of fatty acids in the samples was conducted through a comparison of the starting times of the fatty acid methyl esters standards. The results were expressed as a percentage of the total gas chromatography area.

2.10. Statistical analysis

The means were compared with one way ANOVA and the differences between the means

were significant with P<0.05. The p- and F-values from the one-way ANOVA testing are presented. Tukey's test for post hoc analysis was applied (SPSS 2010). Principal component analysis (PCA) was employed for the purpose of visualizing and interpreting the multivariate relationships between fatty acid composition and the experimental treatments. PCA was conducted on the fatty acid composition and the content of polyunsaturated (PUFA), monounsaturated (MUFA), and saturated (SFA) fatty acids using the Minitab software (Minitab 21.4.1, Minitab Inc., State College, PA, USA).

3. Results and Discussion

Table 1 provides the chemical composition of larvae under varying doses of SiO_2 NPs, highlighting significant changes in key nutritional components. According to the statistical results, when protein content for *G. mellonella* larvae fed with SiO_2 NPs were examined, a notable difference was found between the control group and doses of 5000 ppm and above (x^2=65.020, F= 459.572, df=4, P=0.00) (Table 1). Depending on the increasing SiO_2 NPs dose in the diet, the observed reduction in protein content suggests a potential disruption in protein synthesis or increased degradation, likely linked to oxidative stress induced by SiO_2 NPs (Table 1). A similar situation was observed in fat content, and increasing doses of SiO_2 NPs caused a decrease in the fat content of the larvae (x^2=61.708, F= 123.9, df=4, P=0.00).

The reduction in fat content may be indicative of an interference with lipid metabolism pathways, potentially through the inhibition of lipid synthesis enzymes or enhanced lipid oxidation. The carbohydrate content demonstrated a notable dose-dependent decline, which is likely associated with the increased metabolic demand for energy under conditions of oxidative stress (x^2=0.068, F= 8.570, df=4, P=0.018). In comparison to control group, a decrease in the moisture content in groups treated with 500 and 30000 ppm SiO_2 NPs (x^2=179.600, F= 0.643, df=4, P=0.004). Finally, all of the SiO_2 NPs doses increased the ash content in larvae at a statistically significant level (x^2=0.156, F= 38.427, df=4, P=0.010). The larval total fatty acid composition of *G. mellonella* according to experimental groups are given in Table 2. In this study, thirteen different fatty acids ranging from 6 to 22 carbon atoms were identified in all larvae (Table 2). According to the results of fatty acid composition analysis, larvae contained SFA (up to 60%), followed by MUFA (up to 38%), and PUFA (up to 1.5%) (Table 2).

Among the SFAs, palmitic acid (C16:0) was a major fatty acid (up to 46%). Compared with the control group, the palmitic acid significantly increased depending on the increasing SiO_2 NPs (47–55%, respectively) (x^2=25.881, F= 0.643, df=4 P=0.000). Another most abundant fatty acid in larva was oleic acid (C18:1) (up to 29%), and there was a decline in the this fatty acid in larvae exposed to 30000 and 60000 ppm SiO_2 NPs (down to 22%) (x^2=16.345, F= 593.15, df=4, P=0.000). Similarly, compared with the control group, at all doses of the SiO_2 NPs, caused a significant decrease in the content of capric acid (C10:0) (at 500, 5000, and 30000 ppm SiO_2 NPs doses) (x^2=16.345, F= 593.15, df=4, P=0.000), heneicosenoic acid (C21:1) (at all SiO_2 NPs doses (500-60000ppm)), (x^2=1.532, F= 38.758, df=4, P=0.001), behenic acid (C22:0) (at 500 and 30000 ppm SiO_2 NP doses) (F= 46.00, df=4, P=0.000). Conversely, some doses of the SiO_2 NPs resulted in a notable increment in the linoleic acid (C18:2) (at all SiO_2 NPs doses (500-60000ppm)) (x^2=0.059, F= 10.873, df=4, P=0.011) (Table 2). There were no significant increases or decreases in the other identified fatty acids (Table 2).

PCA was conducted to investigate the differences and similarities between the treatment groups, with the fatty acid composition and saturated and unsaturated properties of fatty acids considered (Figure 1).

PCA identified the effect of SiO_2 NPs on fatty acids more clearly. It was concluded that the experimental groups in which SiO_2 NP was not used or used at a dose of 500 ppm (control and Group 1) exhibited similarities in fatty acid profiles, particularly those of C18:1, C21:1, and C14:0 (Figure 1A).

Table 1. Effects of Silicon dioxide nanoparticles (SiO_2 NPs) on chemical parameters in *Galleria mellonella* larvae[x]

Groups	Protein (%)[y]	Fat (%)[y]	Carbohydrate (%)[y]	Water Content (mg)[y]	Ash content (%)[y]
Control	43.38±0.47 [ax]	52.35±0.40 [a]	2.03±0.08 [a]	101.0±6.0 [a]	2.30±0.05 [a]
500 ppm SiO_2 NP	42.55±0.10 [a]	50.97±0.70 [a]	1.74±0.04 [b]	77.0±2.0 [b]	2.67±0.10 [b]
5000 ppm SiO_2 NP	39.37±0.30 [b]	48.43±0.09 [b]	1.58±0.03 [c]	91.5±5.5 [ab]	2.68±0.03 [b]
30000 ppm SiO_2 NP	33.00±0.02 [c]	43.55±0.30 [c]	1.44±0.01 [cd]	82.0±7.1 [b]	2.54±0.07 [b]
60000 ppm SiO_2 NP	30.67±0.18 [d]	38.92±0.71 [d]	1.31±0.04 [d]	93.0±2.3 [ab]	2.78±0.03 [b]

[x]Values are mean ± standard error from triplicate groups.
[a, b, c] Values within a row with different superscripts differ significantly at P<0.0

Table 2. Larval total fatty acid composition of *Galleria mellonella* according to experimental groups[x]

Fatty Acids Methyl Esters (FAMEs, %)		Control	500 ppm SiO_2 NP	5000 ppm SiO_2 NP	30000 ppm SiO_2 NP	60000 ppm SiO_2 NP
C6:0[y]	Caproic acid	1.67±0.03 [a]	1.60±0.02 [a]	1.67±0.02 [a]	1.64±0.08 [a]	1.59±0.01 [a]
C8:0	Caprylic acid	1.10±0.01 [a]	1.09±0.02 [a]	1.11±0.02 [a]	1.12±0.04 [a]	1.11±0.03 [a]
C10:0	Capric acid	0.49±0.04 [a]	0.35±0.02 [b]	0.32±0.02 [b]	0.38±0.01 [b]	0.41±0.03 [ab]
C12:0	Lauric acid	0.91±0.02 [a]	0.86±0.04 [a]	0.92±0.01 [a]	0.88±0.09 [a]	0.93±0.05 [a]
C14:0	Myristic acid	3.85±0.04 [a]	3.84±0.05 [a]	3.75±0.13 [a]	3.81±0.02 [a]	3.75±0.11 [a]
C15:0	Pentadecylic acid	0.91±0.04 [a]	0.87±0.02 [a]	0.86±0.05 [a]	0.89±0.01 [a]	0.86±0.02 [a]
C16:0	Palmitic acid	46.42±0.07 [a]	47.31±0.15 [b]	48.27±0.13 [c]	51.85±0.33 [d]	55.05±0.07 [e]
C16:1	Palmitoleic acid	0.32±0.03 [a]	0.32±0.02 [a]	0.35±0.01 [a]	0.33±0.04 [a]	0.34±0.02 [a]
C18:0	Stearic acid	4.87±0.19 [a]	4.75±0.07 [a]	4.42±0.17 [a]	4.42±0.04 [a]	4.66±0.17 [a]
C18:1	Oleic acid	29.17±0.03 [a]	29.15±0.06 [a]	29.05±0.12 [a]	25.67±0.22 [b]	22.81±0.03 [c]
C18:2	Linoleic acid	1.56±0.07 [a]	1.76±0.08 [b]	1.76±0.02 [b]	1.91±0.03 [bc]	2.01±0.04 [c]
C21:1	Heneicosenoic acid	8.56±0.23 [a]	7.94±0.01 [b]	7.35±0.01 [c]	6.96±0.17 [d]	6.29±0.13 [e]
C22:0	Behenic acid	0.15±0.01 [a]	0.14±0.01 [b]	0.15±0.01 [a]	0.12±0.01 [c]	0.15±0.02 [a]
∑PUFA	Polyunsaturated fatty acids	1.56±0.07 [a]	1.76±0.08 [b]	1.76±0.02 [b]	1.91±0.03 [bc]	2.01±0.04 [c]
∑MUFA	Monounsaturated fatty acids	38.06±0.23 [a]	37.41±0.09 [ab]	36.75±0.11 [b]	32.96±0.36 [c]	29.45±0.18 [d]
∑SFA	Saturated fatty acids	60.38±0.30 [a]	60.83±0.17 [ab]	61.48±0.09 [b]	65.12±0.38 [c]	68.53±0.21 [d]

[x]Values are the average of three replicates.
[a, b, c] Values within a row with different superscripts differ significantly at P<0.05

These similarities explained the observed variance to a significant extent, while the other experimental groups demonstrated notable differences from these control groups. Conversely, it can be stated that in groups 3 and 4, which have the highest SiO_2 NP usage, C16:0 and C18:2 fatty acids are differentiated from other groups by exhibiting higher values. The changes in fatty acids also affected the ratios of SFA, MUFA and PUFA in total fatty acids. Figure 1B illustrates the significant differentiation between experimental groups based on their distinct fatty acid profiles, which serves to confirm the role of SiO_2 NPs in modifying the lipid composition. Consequently, groups 3 and 4 were separated from the other groups. Conversely, Group 2 exhibits partial similarities to the other groups but also displays distinctive characteristics with regard to specific fatty acids (e.g., C18:2).

Proteins consumed through diet are broken down in the gastrointestinal system of humans or animals by enzymes such as proteases and peptidases, and then converted into amino acids, dipeptides, or tripeptides, which are absorbed in the small intestine [11]. The harmful chemicals applied to insects also affect the structure and quantity of synthesized proteins. It is thought that the increased oxidative stress induced by SiO_2 NPs may potentially disrupt mitochondrial function, which could result in impaired protein synthesis and increased proteolytic activity. Specifically, the activities of synthesized enzymes are either increasing or decreasing [33, 34]. The chemical substance used in our study, SiO_2 NPs doses (at doses of 5000 ppm and above) significantly reduced the protein content (P<0.05) (Table 1).

As known, SiO_2 NPs has the potential to damage mitochondria and subsequently facilitate the increased degradation of proteins, nucleic acids and lipids through reactive oxygen species release (ROS) [35].

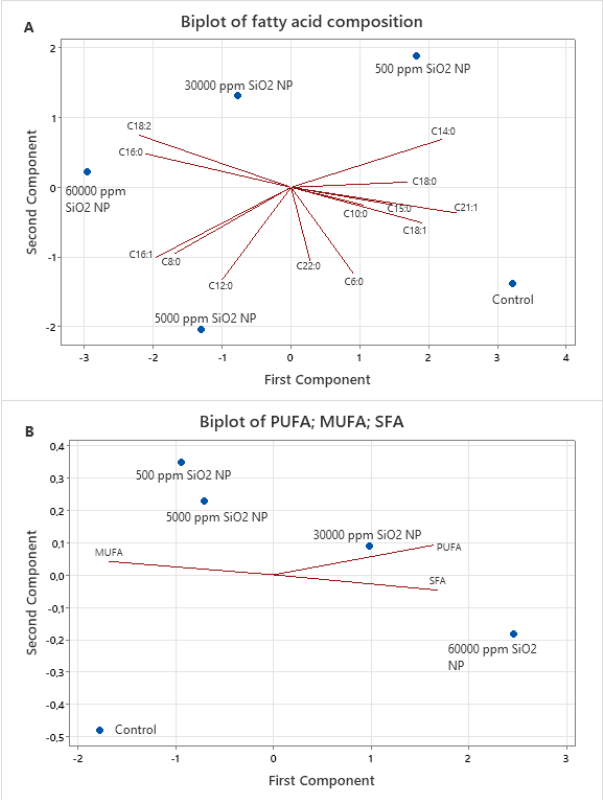

Figure 1. Loading Plot of principal component analysis on fatty acid composition (A), PUFA, MUFA and SFA content (B).

Therefore, exposure of the insect to SiO_2 NPs may have caused oxidative stress that negatively affected its chemical and biochemical parameters (Table 1). Korsloot, van Gestel [36] have noted that stress reactions in insects are known to be energy-demanding processes. It has been argued that energy-demanding stress responses, such as increased repair and detoxification activities, may have contributed to the depletion of protein, fat, and carbohydrate reserves. The organisms may divert energy to repair mechanisms, and pathogenic impacts may cause the depletion of energy stores [37]. Therefore, it is thought that the decrease in protein, fat and carbohydrate contents in *G. mellonella* larvae due to increasing SiO_2 NPs doses is related to NP-induced stress. (Table 1). In addition to all these results, NP-induced stress caused an important reduction in the body moisture content of *G. mellonella* at 500 and 30000 ppm SiO_2 NPs doses as a physiological response ($P<0.05$) (Table 1). It has been reported that loss of moisture content due to external factors may occur as a physiological response in insects [38]. Unlike the moisture content values, all of the SiO_2 NPs doses increased significantly the content of the ash content compared to the control group ($P<0.05$) (Table 1). The decreasing moisture content and increasing ash content have been reported by Markmanuel and Godwin [39] with similar to present study. The elevated ash content indicates the accumulation of mineral content, which may be attributed to disrupted metabolic pathways resulting from NP exposure [39].

The present study provides confirmation of results previously obtained, indicating that the most significant and prevalent fatty acids in *G. mellonella* are palmitic and oleic acids [40, 41] (Table 2). Additionally, larvae contained a significant amount of heneicosenoic acid. However, larvae's contained negligible levels of short-chain and very long-chain fatty acids (Table 2). It is thought that this may be related to feeding of larva's. Kazek, Kaczmarek [41] stated that the presence of short and long-chain fatty acids is related to the diet of the larvae. Furthermore, larvae fed with wax contain more short-chain and long-chain fatty acids.

The findings of studies conducted on *G. mellonella* larvae indicate that alterations in the fatty acid profile may occur as a consequence of oxidative stress, which may be the result of infection, exposure to certain ingredients or different feeding practices [41-44]. It seems probable that this is an adaptive response aimed at counteracting the harmful effects of reactive oxygen species [44]. For example, the changes in fatty acid composition has been evidenced in both LDPE-containing nutrition and *Conidiobolus coronatus* infection [44, 45]. It has been identified that polyunsaturated fatty acids can predispose larvae to oxidative damage, as they are susceptible to ROS attack and can cause to the formation of lipid hydroperoxides [42].

Nevertheless, alterations in fatty acid composition may also be affected by SiO_2 NPs the oxidative stress process within the organism. Indeed, our study demonstrated that the increase in exposure of SiO_2 NPs dose, a source of oxidative stress, resulted in an elevation in

polyunsaturated fatty acids while monounsaturated fatty acids exhibited a decline (Table 2).

Similarly, oxidative stress resulting from various infections has been shown to result in a reduction in MUFA, despite an increase in PUFA [46, 47]. Changes in the fatty acid composition of larvae after exposure to SiO_2 NPs can be attributed to a variety of underlying mechanisms. One of the most fundamental and relevant mechanisms may be oxidative stress caused by SiO_2 NPs. It is well known that NPs can produce ROS when interacting with biological systems [48]. The peroxidation of lipids induced by ROS results in the disruption of membrane integrity, which in turn leads to the degradation of unsaturated fatty acids and alterations in the fatty acid profile [49]. The most well-known consequence of oxidative stress is lipid peroxidation, which causes disruption of cellular membranes and changes in membrane fluidity and function [48].

Furthermore, the potential of SiO_2 to NPs affect lipid metabolism in larvae is also identified. NPs have the potential to inhibit the enzymatic activities involved in the synthesis and degradation of lipids. For example, SiO_2 NPs have the potential to inhibit or alter the activities of desaturase and elongate enzymes, which are essential for the synthesis of long-chain fatty acids. This may result in a reduction or increase in the levels of specific fatty acids, which could lead to alterations in the overall fatty acid profile [49]. Finally, it is hypothesized that SiO_2 NPs may influence the energy metabolism of larvae. NPs have been demonstrated to affect mitochondrial function, which is crucial for energy production and lipid metabolism. This may result in alterations to the synthesis of fatty acids. Hussain, Javorina [50] reported that NPs can disrupt mitochondrial activity, resulting in decreased ATP production and increased fatty acid oxidation as a compensatory mechanism.

4. Conclusion

In conclusion, the exposure of *G. mellonella* larvae to SiO_2 NPs resulted in significant alterations in their chemical parameters, particularly in fatty acid composition. The determined changes can be linked to oxidative stress, interference with lipid metabolism, inhibition of some enzyme activities and mitochondrial dysfunction. These findings highlight the importance of investigating the long-term impacts of nanoparticle exposure on the nutritional quality of edible insects, particularly in the context of their use as sustainable protein sources. The study raises significant questions regarding the safety and regulation of nanoparticles in food systems, emphasizing the requirement for further research and policy development.

Article Information Form

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Authors Contribution
Conceptualization, A.E. and C.O.Ö.; funding acquisition, A.E.; methodology, A.E. and C.O.Ö.; investigation, A.E. and C.O.Ö.; formal analysis, A.E. and C.O.Ö.; writing-original draft preparation, A.E. and C.O.Ö.; writing-review & editing, A.E. and C.O.Ö. All authors have read and agreed to the published version of the manuscript.

The Declaration of Conflict of Interest/ Common Interest
No conflict of interest has been declared by authors.

The Declaration of Ethics Committee Approval
This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics
Authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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