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RESEARCH PAPER

Effect of Titanium Oxide Nanoparticles (TiO_2) on the Gene Expression of Cytochrome P450 Gene in Calliphora vicina Fly Larvae (Diptera: Calliphoridae)

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ABSTRACT

The effect of titanium dioxide (TiO_2) on the gene expression process of the P450 gene was tested in the fly *Calliphora vicina* (Diptera: Calliphoridae). The results showed that TiO_2 was more effective than Delta magic 5% on the gene expression process of P450 gene in species*C. vicina* under laboratory conditions. It was found that there was an increase in the mortality rate significantly to 0.31a ±6.78, 0.43a ± 5.10, 0.48b ± 12.54 and 0.65b ± 14.18 individuals when treated with TiO_2 during (0, 2, 4, 7 days), compared with 0.42a ± 5.99, 0.38a ± 4.13, 0.55ab ± 10.90, and 0.17b ± 14.18, respectively in the control group. It was noticed that there is a difference between the effect of TiO_2 and the pesticide by finding the calculated P value in the statistical tables, since the value of the difference between them in the three concentrations used is P = 0.003, P = 0.1 and P = 0.2, respectively. It was also noted that the significant effect of TiO_2 on the pupation stage of the treated larvae compared with the effect of the insecticide, the highest fail ratio of pupation was recorded, reaching 66% of the total number of treated specimens.

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INTRODUCTION

Calliphora vicina (Diptera: Calliphoridae) and its larvae perform an important ecological function of analyzing the remains of dead animals. They are also widely used in forensic medicine after studying forensic entomology, as a minimum to know when a death occurred. Or the minimum post-mortem period using larval landmarks as "biological timing" [1]. This, in turn, depends on the estimation of the life stages of the corpse-piercing larvae, which usually colonize that corpse shortly after death, because they are often the first colonizers of the corpse. Therefore, its age corresponds precisely to the time of death and provides the most important data [2]. The effect of Nanomaterials on the genetics of this species of insect has been studied, as it is of great importance in the ecological control of nature and urban lands [3]. It has been observed in many studies that considerably changes in environmental conditions, especially temperature, and the effect of widely used pesticides may engender reactive oxygen types. Therefore, understanding the antioxidant system and its molecular mechanisms to defend against damage caused by reactive oxygen types has become a primary focus of research.

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Organisms protect themselves through a group of antioxidant enzymes including cytochrome P450 [4]. Genes (P450) are widely found in almost all organisms and play important roles in insect biology and physiology [5].

Cytochrome P450 monooxygenases are main enzymes that catalyze a diversity of oxidative transformations of both endogenous and exogenous substrates. It arises from the introduction of an oxygen atom bound near the heme iron center of P450 that drives the catalysis, and in insects the P450 belong to four classes and play basic roles in hormone biosynthesis [6]. It also shares resistance to inhibition of types of pesticides and chemical communication [7]. Resistance of insect pests to pesticides is one of the most important reasons for studying cytochrome P450. The objective of this study is to evaluate the effect of different concentrations of titanium dioxide nanomaterial (TiO_2) on the gene expression of Cytochrome P450 gene in *C. vicina* fly larvae and the effect of this on the lifespan of larvae produced after treatment with the nanomaterial.

MATERIALS AND METHODS

Insects rearing

C. vicina larvae were obtained from carcasses of recent death animals and were reared in glass boxes under controlled laboratory conditions with temperature of 30 ± 1 °C and 75 ± 5% relative humidity in continuous darkness. Relative humidity was preserve using a saturated solution of sodium chloride [8]. After the pupal stage, the adults of the insect (male and female) were used for the purpose of mating and producing larvae that will be used to conduct the experiment.

A certain amount of titanium oxide nanomaterial, TiO_2, used in this study, and the product was purchased by Skyspring Nanomaterials, Inc., Houston, USA. The semilethal concentration (LC50) was also estimated by extracting death rate data after 48 hours of treatment and using statistical probability analysis [9].

Testing the effectiveness of insecticides with nanoparticles

The experiment was conducted to first test the effect of the insecticide on the activity of the P450 gene, Then, testing the effect of three different concentrations (0.100, 0.200 and 0.400 mL/L) of TiO_2 nanoparticles after mixing them with the pesticide secondly on specimens of insect larvae during successive periods (0, 2, 4, 7 days) and monitoring all specimens after 48 treatment and recording The results are in special tables for statistical analysis. By comparing the results of the first and second treatments, the effect of the pesticide on the gene expression process is evaluated. Since the increase in the mortality rate for either of the two treatments means the biological effect of them on the catalytic ability of the P450 gene in the synthesis of hormones, resistance to the pesticide and TiO_2 nanoparticles. The carcasses of mice were placed in three of the boxes in which the flies were rearing after spraying the carcasses with the pesticide, and three other carcasses of mice treated with TiO_2 were placed in three boxes in which the *C. vicina* fly was also rearing, and the females were left to lay eggs and form larvae. Then the resulting larvae were calculated from the two treatments. Each experiment was repeated five times [10]. The number of larvae produced was used as a standard for evaluating the biological effect of the two treatments, as the significant decrease in the ratio of larvae produced after the second treatment is an indicator of the effectiveness of nanoparticles according to [11].

RESULTS AND DISCUSSION

The data confirmed in Table 1 show that under the control of laboratory conditions and the increase in the concentration of TiO_2 the mortality rate increased in the three concentrations with the concentration of the control group to 0.31a ±6.78, 0.43a ± 5.10, 0.48b ± 12.54 and 0.65b ± 14.18 individuals after (0, 2, 4, 7 days) of the transaction and respectively. When compared with the effect of the pesticide Delta magic 5%, the ratio reached 0.42a ± 5.99, 0.38a ± 4.13, 0.55ab ± 10.90 and 0.17b ± 14.18, respectively. Through the result of that comparison, it was found that the cumulative mortality rate when treated with nanomaterial and pesticide amounted to 29.84% and 11.43%, respectively. The value of the semi-lethal concentration (LC50) was 0.12 and this confirms the significant effect of the nanomaterial, and this is consistent with Morgen and Trumble [12]. Which indicates that increasing the concentration of TiO_2 leads to an increase in the death rate due to the loss of the ability to resist the impact of that substance. As its effect on the gene expression process of the gene

p450 and consequently the loss of this gene the ability to inhibit the effectiveness of the pesticide and the nanomaterial, as well as a significant decrease in the movement of the larvae formed after treatment. Table 2 shows the calculated P value and its comparison with its counterparts in the Duncan test statistical tables at the probability level ($P < 0.05$)) for the pesticide and TiO_2, and thus knowing the difference between them in terms of the effect on the larvae of species*C. vicina* and for three different concentrations of those substance which are 0.100, 0.200 and 0.400 mg / liter. When comparing the effect of the pesticide and titanium dioxide on the insect larvae and in each concentration, it was found that the value of the difference between them P = 0.003, meaning that TiO_2 is more effective than the pesticide, while the difference in effect between them in the case of the second concentration was little, as it was found that it is equal to P = 0.1, while in the case of comparison between them when treated with the third concentration, it was found that it increased by a small percentage, reaching P = 0.2. It was also observed the significant effect of the nanomaterial on the ability of the larvae group treated, Table 3 shows the ratio of pupa formation and reduction of larvae treated with TiO_2 and pesticide only and for the same concentrations. In the case of the pesticide treatment, it was found that the percentage of pupa formation for the three concentrations with the control group in it was 40b% of the total specimen, whereas, the fail of the formation of the pupa was equal to 0%, that is, it did not reduce any of it, which confirms that it was not affected by the treatment. In the case of concentration 0.100, the rate of pupa formation of the treated larvae was equal to 30a%, and their formation fail was 20%, meaning that there is a significant difference between them at the probability level ($P < 0.05$) which is evidence that they are not affected. As for treatment with a concentration of 0.200 mg/L for each nanomaterial and for the same number of specimens of the two species, a very large significant difference was found between the processes of pupa formation and reduction, as the pupation rate was equal to 28a% of its total, while the fail rate of pupation was 36%, meaning that none of the nanomaterial and the pesticide had any effect on the larvae treated with them and when treating the same number of larvae for both species at a concentration of 0.400 mg/L, the result was the effect of the Nano-material clearly and in a large percentage on the specimens of the two species, as the percentage of pupa formation for the treated specimens in both species was 0.7 cb of the total. While the fail rate of pupation was less than 66%, which is evidence of the significant effect of TiO_2 and the pesticide on the larval role in relation to the trait of pupation in the larvae treated with them. This effect was on the respiratory openings of the larvae in the adult stage (the third stage), which led to their suffocation and thus an increase in their mortality rate. This indicates that the effect of Nanomaterials increases with increasing concentration and thus leads to a loss of the ability of the P450 gene, which the first responsible for the secretion of bio-hormones and protection of the insect body by resisting or inhibiting the effectiveness of any insecticide. It was also found

Table 1. Effect of different concentrations of TiO_2 nanoparticles on the cumulative mortality rate of *Callipora vicina* larvae.

Cumulative mortality of larvae treatment with TiO_2 nanomaterial (Mean ± SE)	Cumulative mortality rate of treated larvae treatment with pesticide (Mean ± SE)	concentrations M/L
6.78 ± 0.31a	5.99 ± 0.42 a	0.00
5.10 ± 0.43 a	4.13 ± 0.38 a	0.100
12.54 ± 0.48b	10.90 ± 0.55 ab	0.200
14.18 ± 0.65 b	14.18 ± 0.17 b	0.400

Table 2. The difference between larvae treated with pesticide and TiO_2.

the difference	TiO_2	Delta magic 5%	Concentrations M/L
P=0.003	0.52	0.44	0.100
P=0.1	0.55	0.53	0.200
P=0.2	0.73	0.69	0.400

Table 3. The percentage of pupa formation and reduction of *Calliphora vicina* larvae treated with TiO_2 and pesticide.

reduction	pupation	ConcentrationsM/L
0.00	0.40 ab	0.00
0.20	0.30 a	0.100
0.36	0.28 a	0.200
0.66	0.7 cb	0.400

that the larvae that formed after treatment with the pesticide only formed the pupae and most of them did not show evident morphological distortions. While the larvae treated with TiO_2 nanomaterial suffered from stunted growth and a significant decrease in motility and ability to be able to pupate, the major influence on the gene expression process of the p45s gene is probably caused by [13]. Based on our studies, we conclude that the larvae are highly dependent on the functional activity of the p450 gene in resistance to the effect of pesticides, bio-hormone synthesis, and the transition from one stage to another. Several studies have been conducted in this field, including the study conducted by Rouhani et al. [14] to find out the effect of silver and zinc nanoparticles on the life roles of the insect *Aphis nerii* (Hemiptera: Aphididae), where the researchers used three different concentrations for each substance, which are 0.13, 424.67 and 539.46 mg / liter, respectively. The results of the study, after conducting a statistical analysis of the treatment data, showed that the effect of $AgNO_3$ nanoparticles is higher than that of ZnO, meaning that there are significant differences between the two substances in terms of the effect that led in addition to the increase in the rate of mortality larvae, pupae and adults of the species, in addition to the fact that the productivity characteristic of female insects decreased by a large percentage when treated with silver than it was when treated with zinc. Sabbour and Shaurub [15] also studied the toxic effect of Imidacloprid and Nano-Imidacloprid molecules after mixing with nanomaterial in controlling *Bactrocera oleae* in laboratory and field working conditions, The results of the statistical analysis in this study using Duncan's test after treating larvae, pupae and adults with seven different concentrations of the two substances, which are respectively 0.125, 0.250, 0.500, 0.750, 1,000, 1,500 and 2,000 mg/l, indicate that the mortality rate increases in the case of treatment. Using the pesticide mixed with the nanomaterial compared to using the pesticide only. As it was found that there are significant differences in the results of the treatment for both of them, and the study confirmed that the ratio of mortality increases with increasing the concentration of the pesticide only and also the pesticide mixed with the nanomaterial. Also, the fertility characteristic of the whole olive fruit fly decreases greatly when treated with Nano-Imidacloprid and compared to using the pesticide Imidacloprid only.

CONCLUSION

In conclusion, the present study demonstrates the significant impact of titanium dioxide (TiO_2) nanomaterials on the mortality and developmental outcomes of the larvae of C. vicina when compared to traditional pesticides such as Delta Magic 5%. The experimental data reveal a clear trend: as the concentration of TiO_2 increases, so does the mortality rate among the larvae, with an LC50 value of 0.12 indicating potent toxicity. The findings suggest that TiO_2 not only enhances mortality but also disrupts the physiological processes related to pupation, significantly affecting the expression of the p450 gene critical for insect resilience against pesticides. Statistical analyses further confirm the superior effectiveness of TiO_2 over the pesticide at various concentrations, underscoring its potential as a more effective biocontrol agent. The adverse effects on larval motility and development, particularly at higher concentrations of TiO_2, highlight the need for further exploration of the mechanisms underlying these changes, particularly in relation to gene expression.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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