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Effects of Engineered Carbon and Silver Nanoparticles on Plutella Xylostella Consumption, Growth, Pupation, Survival, and Fecundity

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EFFECTS OF ENGINEERED CARBON AND SILVER NANOPARTICLES ON *PLUTELLA XYLOSTELLA* CONSUMPTION, GROWTH, PUPATION, SURVIVAL, AND FECUNDITY

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By

Taiaba Afrin

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ABSTRACT

Engineered nanoparticles (ENPs) have recently become common in consumer products, and will therefore be entering into both aquatic and terrestrial systems. However, the effects of ENPs on animals is not well characterized. For example, to date there has been no research on the effects of single walled carbon nanotubes (SWCNTs) and silver nanoparticles (AgNPs) (common ENPs) on insects. I examined the effects of pure SWCNTs and AgNPs on consumption, growth, food conversion ratio, pupation, survival, fecundity, and expression of CYP6BG1 using fourth instar of diamondback moth (DBM) larvae with the following concentrations: 8.64, 17.28, 34.56, 69.12 and 138.24 µg/ml SWCNTs; and 4.32 and 8.64 µg/ml AgNPs. I measured CYP6BG1 expression with Real-time PCR. There were no measurable effects of SWCNT on DBM's consumption, pupation, and survival rate, but negative affect on growth, conversion rate and fecundity at the highest concentration (138.24 µg/ml). AgNPs had no effect on DBM growth, conversion, survival, and fecundity rate, but reduced consumption and pupation rate. In my experiments, CYP6BG1 was upregulated at concentrations of 138.24 µg/ml SWCNTs and 4.32 and 8.64 µg/ml AgNPs compared to control. My research indicates that there is no acute toxicity of SWCNTs on DBM, however further studies are warranted as fecundity and CYP6BG1 expression level appear to be affected. AgNPs are acutely toxic in terms of consumption, but studies are needed to confirm the hypothesis. My research provides insights and a model system for future studies on the effects ENP on insects.

KEYWORDS: *Plutella xylostella,* SWCNT, AgNP, consumption, growth.

This abstract is approved as to form and content

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Dr. D. Alexander Wait
Chairperson, Advisory Committee
Missouri State University

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TABLE OF CONTENTS

Introduction.....1
1.1. Introduction to nanotechnology and nanoparticles……..……..1
1.2. Carbon Nanotubes…….……2
1.3. Silver Nanoparticles…….……4
1.4. Neutral Effects of SWCNT on Animals…..……..…….……..6
1.5. Negative Effects of SWCNT on Animals…….…..…...……8
1.6. Neutral Effect of AgNPs on Animals……..…..……..…11
1.7. Negative Effects of AgNPs on Animals…….……..…11
1.8. Measurement of Gene Expression of CYP6BG1 …….……...…14
1.9. Research Goals and Questions…….……16

Materials and Methods.....18
2.1. Single Walled Carbon Nanotube Properties……..……...…18
2.2. AgNPs Properties…….…….. 18
2.3. Artificial Feed Preparation…….…..……19
2.4. DBM Rearing…….……20
2.5. Artificial Feed Assays…….……20
2.6. Measurement of Fecundity……..……21
2.7. Total RNA Extraction, Purification and Quantitation and cDNA Synthesis…21
2.8. Quantitative Real Time PCR Analysis……..…..22
2.9. Statistical Analysis……...……..…..23

Results ...……25
3.1. Effect of SWCNT on DBM ..…….....25
3.2. Effect of AgNP on DBM……..…28
3.3. Effect of ENPs on CYP6BG1 gene expression level……29

Discussion ……...30
4.1. Effect of SWCNT on DBM……..30
4.2. Effect of AgNPs on DBM……32
4.3. ENPs effect on CYP6BG1 gene expression level……33

References.....……35

Appendix……69

LIST OF TABLES

Table 1. The concentrations of single walled carbon nanotube (SWCNT) and AgNP tested and the number of replicates for each feeding trial.....54

Table 2. Primer design and properties used for qRT-PCR 54

Table 3. Mean (±SE) consumption by diamondback moths (DMB) of feed without and with increasing concentrations of SWCNTs across six trials.…….…… 55

Table 4. Mean (±SE) growth of diamondback moths (DMB) feeding on artificial feed without and with increasing concentrations of SWCNTs……...……...….56

Table 5. Mean (±SE) % of pupation of diamondback moth (DBM) across six different trials for different SWCNT concentration (0 µg/ml, 8.64 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml, and 138.24 µg/ml)……..…….57

Table 6. Mean (±SE) % of survival of DBM across six different trials for different SWCNT concentration (0 µg/ml, 8.64 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml, and 138.24 µg/ml)……..……..……..……58

Table 7. Percentage of µg growth per µg consumption for Diamondback Moth across six different trials. …….....59

Table 8. Number of eggs per adult for Diamondback Moth across three different trials……...……..…..…….……59

Table 9. Table 9: Mean (±SE) data for different variables of Diamondback Moth across six different trials……...…….....60

Table 10. Mean (±SE) consumption rate of AgNPs on DBM across two different trials….....60

Table 11. Mean (±SE) growth rate of Diamondback Moth across two different trials among different AgNPs concentration (0 µg/ml, 4.32 µg/ml and 8.64 µg/ml)…….……..…61

Table 12. Mean (±SE) pupation (%) rate of Diamondback Moth across two different trials…….……..…….61

Table 13. Mean (±SE) survival (%) rate of Diamondback Moth across two different trials……..…….…….61

Table 14. Percentage of µg growth per µg consumption for Diamondback Moth across two different trials among different AgNPs concentration (0 µg/ml, 4.32 µg/ml and 8.64 µg/ml)……..……..…….62

Table 15. Number of eggs per adult for DBM across three different trials among different AgNPs concentration (0 µg/ml, 4.32 µg/ml and 8.64 µg/ml).…….……62

Table 16. Mean (±SE) data for different variables of Diamondback Moth across two different trials....……..…71

LIST OF FIGURES

Figure 1. DBM larvae exposed to SWCNT artificial feed.....…64

Figure 2. Scatter plot of growth/larva (µg) for consumption/larva (µg) of DBM exposed to different SWCNT concentration. 65

Figure 3. DBM larvae exposed to AgNP artificial 68

Figure 4. Scatter plot of growth/larva (µg) for consumption/larva (µg) of DBM exposed to different AgNPs concentration……..…….....69

Figure 5. CYP6BG1 gene expression level for different NPs concentration on DBM……70

INTRODUCTION

1.1. Introduction to Nanotechnology and Engineered Nanoparticles

Nanoparticles that result from human activities (e.g., combustion) or are engineered for consumer products and new technologies are probably encountered by all organisms (Buzea *et. al*. 2007). According to the American Society for Testing Materials, and the Scientific Committee on Emerging and Newly-Identified Health Risks, engineered nanoparticles (ENPs) can be defined as manufactured materials having at least two dimensions between 1−100 nm. ENPs can be categorized into different classes; for example, metals, metal oxides, non-metals, polymer based, functionalized (Klaine *et. al.,* 2008). ENPs can exhibit many novel properties and reactivity because they have high surface to volume ratio compared to other larger sized materials with similar chemical composition (Hochella *et al*., 2008; Auffan *et al*., 2009). ENPs have novel traits in terms of their form and function, unique physical and chemical properties, design, potentially complicated interactions with biological and environmental agents, potential bio-persistence in organisms and feed chains, quick dispensability, bioaccumulation, penetrability through tissue, and irreversible biochemical activities.

ENPs are increasingly used in a wide range of products and technologies; from electronic devices to renewable energy to cosmetics and medicine (Christian, 2009; Fabrega *et al*., 2011). According to, consumer product inventories (CPI) there were 653 products in 2007 containing ENPs but there were 1202 consumer products in 2014 containing NPs (Vance *et al.* 2015). This number is expected to increase significantly

over time. However, there are no data that estimate ENP concentrations or distribution in the environment (Klaine *et al*., 2008).

Because of their novel properties (size, shape, specific surface area, size distribution, chemical composition, and surface structure), some ENPs are thought to be potentially toxic (Sahu and Casciano, 2009; Sharifi *et al*., 2012). Several researchers reported that nanoparticles are more toxic than their counterpart microparticles (Shi *et al*., 2001; Hoet *et al*., 2004; Oberdorster, 2004; Yang and Watts, 2005; Borm *et al*., 2006; Hund-Rinke and Simon, 2006; Powell and Kanarek, 2006). Since ENPs do not have any natural analog, it is difficult to forecast their fate, transport, reactivity, and toxicity in the environmental systems. Therefore, there are concerns about their potential negative effects when released into the environment (Maynard *et al*., 2011; Colvin, 2003; Lowry *et al.*, 2012).

1.2. Carbon Nanotubes

Carbon nanotubes (CNTs) (Iijima 1991) and single walled carbon nanotubes (SWCNTs) (Iijima and Ichihashi, 1993) were developed in 1991 and 1993 respectively. A CPI report (2013) showed that CNTs are the 3rd most common ENP after silver and titanium. CNTs are an allotrope of carbon, and there are three main types of CNTs: single-walled CNTs (SWCNTs), double-walled CNTs (DWCNTs) and multi-walled CNTs (MWCNTs). These types can be defined by their structure and diameter. The synthesis methods for CNTs are mainly carbon-arc discharge, carbon laser ablation, or chemical vapor deposition (CVD). CVD and arc discharge are the most commonly used

method to produce SWCNTs (Cheng *et al*., 2007). Raw CNTs can be purified and functionalized, which may alter their toxicity (Sun *et al*., 2002; Bianco *et al*., 2005).

SWCNTs are developed from a single graphite sheet rolled into a cylindrical tube (Fako and Furgeson, 2009) and have a diameter of 0.7 to 3.0 nm, with the length can be hundreds of micrometers. SWCNTs have extraordinary electrical, mechanical, thermal, optical, and chemical properties (Dresselhaus *et al*., 2001; Baughman, *et al*. 2002; Gracia-Espino *et al*., 2010). They can have metallic, semi-conductive, and superconductive properties and high tensile strength (Avouris, 2002; Baughman, *et al*. 2002). SWCNTs can be found in field-effect transistors (Bradley *et al*., 2003; Snow *et al*., 2003; Meitl *et al*., 2004; Zhou *et al*., 2004), chemical sensors (Maklin *et al*., 2007), nano-electronic devices (Rueckes *et al*., 2000; Bachtold *et al*., 2001; Avouris, 2002), hydrogen storage cells (Dillon *et al*., 1997; Liu *et al*., 1999; Kayiran *et al*., 2004), sorbents for toxic chemicals (Long and Yang, 2001a; Long and Yang, 2001b; Lacerda *et al*., 2006), *in situ* sensing (Kruss *et al*., 2013), imaging (Robinson *et al*., 2012), and for thermal ablation (Kosuge *et al*., 2012). They have a very high surface area which allows for extensive molecular loading and multi-functionalization, as well as efficient internalization into a cell in a large number (Kostarelos *et al*., 2007; Boyer *et al*., 2016). They can also transport proteins, oligonucleotides, long nucleotide chains, (Kam *et al.,* 2006) and drugs to specific sites in organisms (Bianco *et al*., 2005).

Raw SWCNTs are mainly hydrophobic, whereas purified SWCNTs are mainly hydrophilic because of the functional groups on their surfaces (Sun *et al*., 2002). There is controversy about whether purification of raw SWCNT reduces (Sayes *et al*., 2006) or increases (Tian *et al.* 2006) their toxicity; and the effect of functionalized SWCNT on

different animals during their early development is still unknown (Philbrook *et al*., 2011). Various functionalization and dispersion methods, as well as their differential fates, could increase or decrease their toxicity (Kennedy *et al.,* 2009).

Concerns about potential risks of organisms exposed to SWCNTs are also paralleled with their increasing use, disposal, and diffusion into the environment. At present, studies for SWCNTs toxicity are limited (Shvedova *et al.,* 2008; Donaldson *et al*., 2010; Johnston *et al*., 2010; Castranova and Mercer, 2012; Ong *et al*., 2016b). To date, there have been several studies published that examine the toxicity of SWCNTs on vertebrates. On the other hand, very few studies have been conducted on arthropods, yet, arthropods will potentially be exposed to SWCNTs in the environment. Concerns about environmental risks of SWCNTs and considerations about the research on toxicity testing have already been expressed in several reports and review papers (Petersen and Henry, 2011; Du *et al*., 2013). Proactive research for clear understanding about the toxicity of SWCNTs toxicity is increasingly important with their production, use and disposal in ever larger quantities.

1.3. Silver Nanoparticles

Another class of engineered nanoparticle that are becoming increasingly common are Ag nanoparticles (AgNPs). There is interest in their use for antimicrobial (Lee *et al*., 2007a; Demir *et al*., 2011) and anti-inflammatory (Bar-Ilan *et al*., 2009) properties. In addition, AgNPs are effective against multidrug resistance strains of bacteria such as methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa* (Palanisamy *et al*., 2014), ampicillin-resistant *Escherichia coli* O157:H7, and erythromycin-resistant

staphylococcus pyogenes (Shahverdi *et al*., 2007). Consequently, AgNPs have advantages over other antibiotics (Yamanaka *et al*., 2005; Lara *et al*., 2011).

Because of their properties, they are also increasingly incorporated into consumer products such as feed packaging (Edwards-Jones, 2009), deodorants, clothing materials, bandages (Chen and Schluesener, 2008), burn treatments (silver sulfadiazine), socks, soaps and detergents, water and air filters, washing machines, wet wipes, bedding, coating on surgical instruments and medical and industrial textiles (Buzea *et al*., 2007; Chen *et al*., 2007; Kumari *et al*., 2010; Liu *et al*., 2010). They are also used in optical microscopy, surface-enhanced Raman spectroscopy (SERS), nanodevices for biological sensing (Sun and Xia, 2003). AgNPs are part of HIV, ovulation and pregnancy tests (Wagner *et al*., 2006), and used in cancer and tumor detection (Ong *et al*., 2016a). AgNPs can be infused into synthetic tissue adhesives in ophthalmic applications, as they have higher antibacterial properties and greater mechanical strength (Yee *et al*., 2015).

With the use of high quantities of AgNPs, the accumulation in the environment and the exposure of living tissue of AgNPs are increasing (Chen and Schluesener, 2008). There is little information about how Ag from AgNP work inside the cell (Armstrong *et al*., 2013). Some researcher believes that the lethal properties of AgNP to microbial cells are also responsible for their toxicity to eukaryotic cells (Buzea *et al*., 2007). There is some evidence that particle size or surface area (Oberdorster *et al*., 2005; Risom *et al*., 2005; Nel *et al*., 2006; Oberdorster *et al*., 2007; Rogers *et al*., 2007; Jiang *et al*., 2008) is mainly responsible for AgNP toxicity, as Ag^{+} released from the NP surface after oxidation could enter into the body and interact with biological molecules (Moore, 2006; Lin *et al.*, 2010; Park *et al*., 2010). Several researchers found that ionic Ag is highly toxic to

bacteria (Liau *et al*., 1997), phytoplankton (Ratte, 1999), invertebrates, and fish (Croteau *et al*., 2011). It is also known that, AgNPs interact with proteins (in particular with thiol groups) and promote denaturation of proteins (Johnston *et al*., 2010). Ag also interacts with proteins associated with cellular copper homeostasis (Winder and Harris, 1991). Surface coatings, functionalization, and stabilization could also influence their toxicity and biocompatibility (Bar-Ilan *et al*., 2009). For example, Posgai *et al*., (2011) concluded that, smaller and uncoated particles were more toxic than larger and coated particles respectively.

1.4. Neutral Effects of SWCNT on Animals

There are a number of studies examining SWCNTs that have not found any measurable impact on animals. Lung tissue in rats exposed to 0.03 or 0.13 mg/m^3 SWCNTs in the air was not negatively affected (Morimoto *et al*., 2012). Rats injected with SWCNTs did not show any abnormality in cardiac function (Joviano-Santos *et al.* 2014). Mice aspirated with SWCNTs at 40 µg were similarly not negatively affected (Kagan *et al*., 2010). Finally, Philbrook *et al*. (2011) reported that there were no effects on litter sizes, maternal weight, fetal lengths, weight, and viability in pregnant mice exposed to 10 mg/kg SWCNTs with >90% purity and functionalized with a hydroxyl (–OH) group.

SWCNT contamination in Rainbow trout (*Oncorhynchus mykiss*) blood (in red and white blood cell counts), haematocrit, whole blood hemoglobin did not cause negative effects, and; tissue metal levels and ATPase activity in brain were also unaffected (Smith *et al.,* 2007). Fraser *et al.* (2011) reported no significant impact of 500

mg/kg SWCNTs on Rainbow trout mortality, consumption, growth, plasma protein, or ATPase activity in the gill, intestine, or brain. Cheng *et al*. (2007) reported no significant negative effects of 240 mg/L SWCNT solution on zebrafish on head trunk, body length, cell death, regulatory factors (myogenic, notochords and primary sensory neurons), pigment development, main blood vessel formation, or hatching success and survival rate. Templeton *et al*. (2006) found no negative effects on developmental, fertilization success, life-cycle mortality and growth rate in estuarine copepods *Amphiascus tenuiremis* exposed to 10 mg/L purified SWCNTs. *Daphnia magna* (48 h of exposure) and medaka fish *Oryzias latipes* (96 h of exposure) exposed to raw SWCNTs (upto 100 mg/L) did not show any mortality and immobility (Sohn *et al*., 2014).

The number of SWCNT toxicity studies conducted on insects is very limited. *Drosophila melanogaster* supplemented with SWCNT feed (1000 µg/g) did not show any detectable toxicity on egg to adult survivorship, rather SWCNTs were sequestered in tissue (Liu *et al*., 2009). No significant effect was observed on *D. melanogaster* fed on ~10 ppm of disaggregated SWCNT on their growth, viability, fertility, but SWCNTs was incorporated into tissues of the larvae (Leeuw *et al*., 2007). Lee *et al*., (2015) found that a 100 µg/ml SWNT had no effect on larval viability, lifespan, fecundity, pupal viability, physical activity of female flies and resistance to starvation stress on *D. melanogaster* larvae fed with carbon nanofiber, CNF. *D. melanogaster* 0.5%, w/v SWCNTs with >90% purity and functionalized with a hydroxyl (–OH) group showed no significant effect on fecundity, egg viability, developmental stage, and histology (Philbrook *et al*., 2011).

1.5. Negative Effects of SWCNT on Animals

Manna *et al*. (2005) found that human keratinocyte cells exposed to 10 µg/ml SWCNTs exhibited increased oxidative stress and cell proliferation inhibition. Cui *et al*. (2005) reported that, 25 µg/ml SWCNTs decreased tyrosinase activity (responsible for pigment development) in human embryonic kidney cells (HEK293 cells). Choi *et al*. (2009) reported increased inflammatory responses, and elevated oxidative stress and cell membrane damage and Kisin *et al*. (2007) reported reduction of viability and induction of DNA damage in lung fibroblast (V79) due to SWCNT exposure. SWCNTs caused bundle formation of actin filaments as well as impairment in proliferative activity (Holt *et al*. 2010), induction of DNA damage due to incorporation into centrosome structure (Sargent *et al.* 2009), formation of stable protein complexes and disruption of protein structure (Zuo *et al*., 2010), and damaging of ion channel function due to changes in proteins (Park *et al*., 2003).

Legramante *et al*. (2009) reported decreased heart rate in rats due to SWCNTs. Mice intratracheally instilled with 0, 0.1, or 0.5 mg of SWCNTs for 7 days induced dose dependent epithelioid granulomas and interstitial inflammation (Lam *et al*., 2004). Mice that inhaled 5 mg/m^3 SWCNTs produced elevated inflammatory responses, collagen deposition, oxidative stress, and fibrosis in the lungs (Shvedova *et al*., 2008). Cardiopulmonary toxicity was reported for mice exposed to aspirated 10 µg or 40 µg SWCNTs (Tong *et al*., 2009). Teeguarden *et al*. (2011) reported that mice aspirated with 40 µg SWCNTs twice a week produced severe inflammatory and fibrotic responses. Shvedova *et al*. (2014) found that mice that inhaled 5 mg/m^3 pure SWCNTs showed histopathological changes in the lungs and tracheobronchial lymph. Philbrook *et al*.

(2011) reported pregnant mice exposed to 10 mg/kg SWCNTs with >90% purity and functionalized with a hydroxyl (–OH) group showed higher percentage of resorptions, gross morphological defects, skeletal abnormalities, aberrations in tail structure, defects in cervical vertebrae and variable ossification of sternebrae. SWCNTs induced dose dependent cytotoxicity in alveolar macrophages of guinea pig (Jia *et al*., 2005).

Smith *et al*. (2007) reported dose dependent effects in ventilation rate, gill pathologies (oedema, altered mucocytes, hyperplasia), mucus secretion and increased ATPase activity of gills and intestine in Rainbow trout (*O. mykiss*) exposed to SWCNTs. Rainbow trout supplemented with 500 mg/kg SWCNTs diet showed significant elevation in brain TBARS (an indication of lipid peroxidation) (Fraser *et al*., 2011). SWCNTs caused hatching delay in zebrafish embryos exposed to 120, 240, and 360 mg/L with no obvious dose response (Cheng *et al*., 2007).

Two freshwater microalgae (*Raphidocelis subcapitata* and *Chlorella vulgaris*) showed reduced growth rate and biomass exposed to raw SWCNTs (upto 46.10 mg/L) (Sohn *et al*., 2014). SWCNTs were reported to effect ingestion and digestion of bacteria (Ghafari *et al*., 2008), induction of toxic effects on the development of *Escherichia coli* (*E. coli*) biofilm (Rodrigues and Elimelech, 2010) and bacterial cell death due to SWCNTs aggregation (Kang *et al*. 2007).

Petersen *et al*. (2008) reported the accumulation of C-labeled SWCNT in the gut of *Lumbriculus variegatus*, 80% of SWCNTs were purged after 3 days. Mwangi *et al*. (2012), reported that 1 g/L SWCNT reduced the survival rate of an amphipod (*Hyalella azteca*), a midge (*Chironomus dilutus*), and an oligochaete (*Lumbriculus variegatus*). They also reported accumulation of SWCNTs on their gut. 10 mg/L complex as prepared

SWCNTs slowed development, reduced fertilization success and increased mortality of estuarine copepods *Amphiascus tenuiremis* (Templeton *et al*., 2006). The arthropod *D. magna* showed reduced immobilization and increased mortality when exposed to 100 mg/L SWCNTs (purity > 60%) (Zhu *et al*., 2009). Roberts *et al.* (2007) reported that lipid-coated SWCNTs accumulated in the gut *D. magna*, and the animals suffered acute toxicity (reduced survival rate and increased mortality) as a result of exposure at levels up to 2.5 mg/L.

There is very little information published, however, on the effects of CNTs on insects. SWCNT reduced climbing activity, overwhelmed the natural grooming mechanism, impaired locomotor function, and increased mortality rate in *D. melanogaster* (Liu *et al*., 2009). Lee *et al*. (2015) reported that *D. melanogaster* larvae fed on 1000 μg/ml CNF had reduced larval viability, lifespan and fecundity. Male flies also had reduced physical activity. They also found that *Drosophila* larvae developed resistance against starvation stress, generated increased number of crystal cells (A type of blood cell produced when flies face any immune challenge), and reactive oxygen species.

1.6. Neutral Effect of AgNPs on Animals

Sprague-Dawley Rats that inhaled AgNPs (1.32×106 particles/cm^3,61 μg/m^3) for 28 days had no changes feed consumption, body weight, organ weight, hematology or blood biochemical values. However, AgNPs were detected in liver and heart tissue in a dose dependent manner (Ji *et al*., 2007). Feed and water consumption, and female body weight of F344 rats fed was not affected by 56 nm AgNPs (up to 500 mg/Kg) in their feed (Kim *et al*., 2010).

Lim *et al*. (2012) found no adverse effect in *C. elegans* on pmk-1 (km25) mutant in case of ROS formation and HIF-1 and GST activation and reproduction rate after exposure to AgNPs. Zhao and Wang (2011) found that, 500 µg/ml AgNPs are not acutely toxic to *D. magna*. They also reported no significant toxic effect of AgNPs on mortality, but very high amount of accumulated AgNP in gut linings. Kim *et al*. (2011) reported that, AgNPs suspensions excluding Ag^+ were not acutely toxic to *D. magna.*

Silver-Key *et al*. (2011) reported that 5%/ ~9000 ppm AgNPs did not affect *D. melanogaster* hatching rates and that 50 mg/L of AgNPs did not affect adult survival, metamorphosis, and lifespan of *D. melanogaster* (Armstrong *et al*., 2013).

1.7. Negative Effects of AgNPs on Animals

There are a handful of studies that have found AgNPs toxicity to vertebrates. Haase *et al*. (2011) reported AgNPs strong cytotoxicity and cell death on human macrophages (monocytic leukemia cell line THP-1) in dose and time dependent manner. They also concluded that the smaller AgNPs are more toxic than larger ones.AgNPs caused a significantly higher amount of free radical generation and glutathione content reduction in BRL 3A rat liver cells (Hussain *et al*., 2005). Ahamed *et al*. (2008) reported DNA damage and apoptosis in mouse embryonic stem cells and fibroblasts. Kim *et al*. (2008) found significant dose-dependent changes in rats for alkaline phosphatase activity, cholesterol level and liver function. Tang *et al*. (2008) reported accumulation of AgNPs into rat brain after 62.8 mg/kg AgNP injection and subsequent neuronal degeneration and necrosis. 56 nm AgNPs (up to 500 mg/Kg) fed to F344 rats resulted significant decrease in male body weight, slight liver damage and higher incidence of bile-duct hyperplasia

(Kim *et al*., 2010). Rahman *et al.* (2009) found that 25 nm sized AgNPs (up to 1000 mg/kg AgNP) induced neurotoxicity in male C57BL/6N mice by altering gene expression, generating oxidative stress, and inducing apoptosis. AgNPs were found to inhibit the proliferation of mouse spermatogonial stem cells through blocking GDNF/Fyn kinase signaling (Braydich-Stolle *et al*., 2010). Significant negative effects of AgNPs in rates were observed for spermatogenic cell number (Miresmaeili *et al*., 2013), sperm abnormalities (Mathias *et al*., 2014), and spermatocyte development (Han *et al.,* 2016).

Citrate capped AgNPs (0.04–0.71 nM) transported into the zebrafish embryo induced deformities and finally death (Lee *et al*., 2007b). Bar-Ilan *et al*. (2009) reported that zebrafish exposed to 250 μM AgNPs had decreased survival rates and suffered the following abnormalities: stunted growth, opaque and nondepleted yolk, small head, jaw, snout, tail and circulatory malformations, and body degradation. Choi *et al*. (2010) reported that AgNPs at a concentration of 250 mg/L AgNPs was lethal to zebrafish. Choi *et al*. (2010) also found induction of metal-sensitive metallothionein 2 (MT2) mRNA in liver tissue and reduced levels of oxyradical-scavenging enzymes catalase and glutathione peroxidase 1a mRNAs. They also found such cellular alterations as disruption of hepatic cell cords and apoptotic changes and increased level of malondialdehyde (a byproduct of cellular lipid peroxidation) and total glutathione. They also documented increased DNA damage and upregulation of the *Bax, Noxa*, and *p21* genes. Yoon *et al.* (2007) demonstrated that AgNPs were toxic to bacteria.

Ali *et al*. (2014) reported lethal (48.10 μg/L) and sublethal (36 μg/L) toxicity of AgNP in the fresh water snail *Lymnaea luteola L*.; at sublethal concentration, AgNPs caused higher lipid peroxidation, catalase activity in the digestive gland, DNA damage,

and reduced glutathione level, and increases in glutathione peroxidase and glutathione-S-transferase activities. Meyer *et al*. (2010) reported AgNPs to be toxic to *C. elegans*. Kim *et al*. (2012) found reduced survival and reproduction, generation of severe epidemic edema and burst (even though there were no evidence of AgNP intake) in *C. elegans* exposed to citrate-coated AgNPs (up to 100 mg/L). *C. elegans* increased ROS formation, expression of PMK-1 p38MAPKand hypoxia-inducible factor (HIF-1), GST enzyme activity and decreased reproduction in wildtype (N2) after exposure to AgNPs (Lim *et al.,* 2012). Zhao and Wang (2011) reported chronic toxicity in *D. magna* exposed to AgNPs. Poynton *et al.* (2012) reported citrate/PVP coated AgNPs toxicity on *D. magna.* They found disrupted major biological process by AgNPs (i.e. protein metabolism, signal transduction); PVP coated AgNPs induced metal responsive and DNA damage repair genes. Citrate coated AgNPs were more toxic than same sized PVP coated AgNPs.

D. melanogaster is the only insect that has been experimentally exposed to AgNPs to examine toxicity. Ahamed *et al*. (2010) reported that *D. melanogaster* fed with 100 µg/ml AgNPs responded with the following changes: increased expression of Hsp70, p53 and p38 protein (due to increased DNA damage), induction of oxidative stress and higher activities of caspase-3 and caspase-9 (apoptosis). Demir *et al*. (2011) reported that AgNPs (10 mM) induced genotoxicity of *D. melanogaster* larvae. Posgai *et al*. (2011) reported negative, dose dependent (up to 200 µg/ml) effects of AgNP on *D. melanogaster* survivorship, pupation, mating success, and abnormalities in cuticular and melanization development. Silver-Key *et al*. (2011) reported impaired larval developmental progression, reduced body pigmentation, shortened life span, abnormal climbing behavior and stress induction in *D. melanogaster* larvae fed with 5%/ ~9000 ppm AgNPs. *D.*

melanogaster showed demelanized adult cuticle, reduced progenies, verticle movement, tyrosinase and Cu-Zn superoxide dismutase activity when feed was supplemented with 50 mg/L of AgNPs (Armstrong *et al*., 2013). Ong *et al*. (2016a) reported accumulated AgNPs in *D. melanogaster* gut, reduced viability, slower developmental process, reduced fecundity of males and increased level of ROS at the apical tip of the testis when fed with 5 mg/L AgNPs.

1.8. Measurement of Gene Expression of CYP6BG1

The cytochrome (CYP) P450 monooxygenases (p450s) are an abundant gene superfamily of heme-thiolate proteins, and this group of enzymes is found in almost all living organisms (Werck-Reichhart and Feyereisen, 2000). CYP genes can be categorized into 4 major clans; CYP2, CYP3, CYP4 and mitochondrial (Nelson, 1998). They are involved in the first step of drug metabolism, in detoxification of numerous xenobiotics and endogenous substances, and are essential for proceeding to second step of detoxification (Pelkonen *et al*. 1998; Martignoni *et al*. 2006; Fröhlich *et al*., 2010).

In an *in vitro* study Sereemaspun *et al*. (2008) reported inhibition of CYP1A2, CYP2C19, and CYP3A4 in heterologously expressed human p450s in insect cell membrane exposed to 15 nm sized AgNPs. In Rainbow trout, 10 nm sized AgNPs caused CYP1A2 induction in gill tissue (Scown *et al*., 2010). Kulthong *et al*. (2012) found no significant effect of orally administered AgNPs (~180 nm diameter; up to 1000 mg/kg) on CYP activities in vivo in Sprague-Dawley rat, but reported inhibition of CYP2C and CYP2D activities *in vitro*. SWCNTs inhibited CYP3A4BR activity in a dose-dependent

manner by choking the exit channel of substrate/products through a complex mechanism in Bactosomes (El-Sayed *et al*., 2016).

The CYP3 clan is the largest clan (Cui *et al*., 2017) incorporating with CYP6 and CYP9 gene families. They are found among insect p450 genes in large clusters (Feyereisen, 2006). Gene families from this clan play very important roles in insects via inactivation and metabolism of xenobiotic compounds such as insecticides and pesticides (Scott, 1999; Feyereisen, 2005; Bernhardt, 2006; Iga and Kataoka, 2012; lin *et al*., 2013). Genes from this clan are referred to as "environmental response genes" (Berenbaum, 2002).

Resistance to toxic chemicals in insects are often associated with one or more detoxifying genes; e.g., p450s, esterases and glutathione S-transferases (Matambo *et al*. 2010; Stevenson *et al*. 2011; Niu *et al*. 2011; Martinez-Paz *et al*. 2012). CYP6B enzymes are believed to be mostly responsible to insecticides in caterpillars (Cohen *et al*., 1992; Berenbaum *et al*., 1996). In several insects, a large number of CYP6 genes have been identified, which are associated with toxic chemical resistance. For example, CYP6A1 (Carino *et al*., 1992; Carino *et al*., 1994), CYP6D1 (Tomita *et al*., 1995; Liu and Scott, 1998) and CYP6A12 (Guzov *et al*.,1998) in housefly; CYP6CM1 (Karunker, *et al*., 2008) in whitefly; CYP6CY3 (Puinean, *et al*., 2010) in peach aphid; CYP6B3, CYP6B4, CYP6B5 (Scott and Wen, 2001) in butterfly *Papilio polyxenes*; CYP6BQ9 (Zhu *et al*., 2010; Zhu *et al*., 2013) in red flour beetle; CYP6BQ23 (Zimmer *et al*., 2014) in pollen beetle; CYP6ER1 and CYP6AY1 (Bass *et al*., 2011; Ding *et al*., 2013; Bao *et al*., 2016) in brown planthopper; CYP6G1 (Daborn *et al*., 2001; 2008; Hoi *et al*., 2014) in *D. melanogaster*; CYP6AB11 (Niu *et al*., 2011) in *Amyelois transitella*; CYP9G2 (Shen *et*

al., 2004) and CYP6BF1 (Li *et al*., 2005) in diamondback moth (DBM). More than half of CYP P450 genes were upregulated in the resistance strain of Colorado potato beetle against imidacloprid pesticide (Zhu *et al*. 2016). Bautista *et al*. (2007) reported that, permethrin resistance at the fourth instar stage of DBM larvae was associated with CYP6BG1 overexpression in resistant strains and inducible in their susceptible counterpart. Bautista *et al*. (2009) confirmed that CYP6BG1 overexpression was due to increased metabolism for permethrin detoxification through RNA interference mediated gene silencing (RNAi).

Only few studies examined whether the CYP p450 gene is expressed differentially in animals exposed to, presumably, toxic concentrations of ENPs. El-Sayed *et al*. (2016) examined the effect of carboxylated SWCNT on animal CYP activity. Fröhlich *et al*. (2010), Lamb *et al*. (2010) and Warisnoicharoen *et al*. (2011) examined the effects of AgNPs on CYP activity.

1.9. Research Goals and Questions

I examined the effects of these ENPs on DBM because it is considered a model pest. It is a major pest on cruciferous crops (Talekar and Shelton, 1993) and can migrate and reproduce very quickly (Yu *et al*., 2015). It is remarkably resistant to insecticidal toxins (Sun *et al*., 1986; Sun, 1992; Talekar and Shelton, 1993; Scott and Wen, 2001; Furlong *et al*., 2013), and toxicity to ENPs would indicate that many insects might be negatively affected. For example, DMB was the first pest that became resistant to DDT (Ankersmit, 1935; Jhonson, 1953). In almost all countries they have evolved resistance

against agricultural synthetic insecticides (Talekar *et al*., 1990), even the bio-insecticides based on *Bacillus thurengiensis spores* (Tabashnik *et al*., 1997).

The goal of my research was to examine if SWCNTs or AgNPs are toxic to an insect herbivore, the DMB. The first question I addressed was whether these two ENPs were a deterrent to DBM feeding. I hypothesized that these ENPs do not deter feeding as the larvae consumed ENP feed. The second question I addressed was whether these ENPs had an effect on the amount of feed DMB consumed? I then examined if consumption of ENPs affected growth. For example, if they consume the same amount of control feed and feed with ENPs, will the larvae grow more or less? I then related ENPs consumption and growth rate to survival rates, pupation rates and fertility (number of eggs laid). Finally, my research examined if there was any evidence for detoxification of the ENPs? Specifically, were p450 genes upregulated or downregulated?

MATERIALS AND METHODS

2.1. Single Walled Carbon Nanotube Properties

SWCNTs (COOH-CNT) produced by the catalytic vapor deposition (CVD) process were purchased from NanoLab Inc. (Waltham, MA, USA) in powdered from. For this study, they were dispersed in 18MΩ deionized water to make the desired concentration (290 mg/L or 292 mg/L or 305 mg/L) with more than 95% purity and pH of 6.5 -7.5. Initially, 400 mg of SWCNT material were added to 1L of 18MΩ deionized water. A probe sonicator was used for dispersion (twice for 30 min with a 15 minutes' rest in between) followed by ultracentrifugation at 25,000g for 30 min. The supernatant collected from the centrifuge tubes and ultracentrifugation was repeated at 25,000g for 30 minutes. During this process, the concentration decreases due to the removal of amorphous carbon and unfunctionalized carbon material and finally yield an approximate 300 mg/L desired concentration. The SWCNTs have an approximate diameter of 1.5 nm, length of 1-5 μm and surface are of 1020.48 m^2/g.

2.2. AgNPs Properties

AgNPs (20 nm PELCO® citrate NanoXact™ AgNPs) were commercially obtained from Ted Pella Inc. (Redding, CA, USA). Their diameter was 18.5 ± 3.4 nm and their hydrodynamic diameter was 28 nm with 29.0 m^2/g surface area (TEM). The concentration of the AgNP suspension was 0.021 mg/ml, with a particle concentration of 6.0E + 11 particle/ml. The AgNP has -43 mV zeta potential and 396 nm absorbance peak (λ_{max}) with 3.50 max Optical Density/cm in a solution with 8.1 pH. The particle surface was sodium citrate and the aqueous carrier was 2 mM citrate. AgNPs were stored at 4ºC

and away from light before use and they were used directly without any processing or change before use.

2.3. Artificial Feed Preparation

The lab benches were sterilized and cleaned with 75% ethanol before the preparation of the experimental feed to avoid any infection to DBM larvae through feed with any natural microorganism. The artificial feed was a dry mix purchased from Southland Products Inc. (Lake Village, AR, USA). The mix was specifically formulated for DBM (*P. xylostella*). The recipe for 250 ml feed was as follows: 40.5 g dry mix and 1.75 ml raw linseed oil and 232.5 ml deionized boiling water. For SWCNT feed preparation, 1.75 ml raw linseed oil was added to 40.5 g dry mix in an Erlenmeyer flask. Then SWCNT solution was added in 7.45 ml, 14.89 ml, 29.79 ml, 59.58 ml or 119.2 ml volume to generate 8.64 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml and 138.24 µg/ml SWCNT feed respectively. The mixture was then combined with deionized boiling water (225.05 ml, 217.61 ml, 202.71 ml, 172.92 ml and 113.3 ml respectively) on a magnetic stirrer hot plate. After mixing the final suspension for 2-3 minutes, the resulting semi-liquid feed was poured into labeled Petri dishes and left in room temperature about 15-20 minutes for solidification. The solidified feed mixture was then partitioned using a small corer (1.3 cm diameter) and the remaining feed was stored in the refrigerator at 4ºC.

To prepare 60 ml AgNP feed, 0.42 ml raw linseed oil was added to 9.72 g dry mix in a flask. Then AgNP solution was added in a volume of 12.34 ml and 24.69 ml to achieve a final concentration of 4.32 µg/ml and 8.64 µg/ml AgNP feed respectively. It was then combined with deionized boiling water (43.46 ml and 31.11 ml respectively).

Other steps were the same as SWCNT feed preparation. Nanomaterial free feed were used as control feed. There were also two other "control" arenas: 1) No larvae control (NLC), feed discs from all treatments without any larvae to determine any differences in how fast control and ENP feed discs dry out, 2) No feed control (NFC), starvation arenas with larvae but no feed to determine starvation rates.

2.4. DBM Rearing

Eggs of DBM were purchased commercially from Benzon Research Inc. (Carlisle, PA, USA). Eggs on aluminum foil were kept with artificial control feed discs in plastic feed boxes under diurnal cycle of 16 h light: 8 h dark to hatch and grow until they are 2nd instar larvae. The lab temperature was 25 ± 3ºC with 65 ± 5 % relative humidity. The DBM laboratory is licensed by the United States Department of Agriculture to rear and 3w: P26P-14-02726.

2.5. Artificial Feed Assays

Four artificial feed discs were placed in each petri plates except for the starvation arenas. There were six trials for SWCNT and two trials for AgNP. Details about the treatments and number of replicates are given in Table 1. From the artificial feed, the larvae were provided with 0.0 mg, 51.3 mg, 85.7 mg, 172.0 mg, 294.6 mg, 557.6 mg SWCNT and, 8.2 mg and 15.4 mg of AgNP.

Fifteen DBM larvae were placed into each Petri dish (treatment arena) except for NLC and allowed to consume the feed for about 72 hours (until pupation). Artificial feed

consumption by larvae and larval growth were determined by weighing feed discs and larval weight every 8-12 hours.

2.6. Measurement of Fecundity

To collect the eggs, I used cabbage treated aluminum foil. Foil was cut into 10 cm X 4 cm pieces and straight parallel lines were drawn into the foil with a needle. Then they were dipped into freshly prepared cabbage juice. 26 grams of fresh cabbage were blended with 200 ml distilled water and then strained. The foil pieces were then soaked in the juice and left to dry. This was repeated at least 5 times and finally the foils were left to dry overnight. The foils were folded half (to 5 cm X 2 cm) and hung from the lid of the oviposition container, which were prepared from 5.5 oz. cups.

After the larvae went into pupation, they were transferred into oviposition containers. The containers were supplied with fresh artificial feed after adult emergence. Folded foils with appropriate leveling were hung from the lid to facilitate egg laying. Foils were changed after every 2 days and stored in a refrigerator at 4ºC for about 4-6 days. Eggs were counted after 9/10 days of adult emergence.

2.7. Total RNA Extraction, Purification and Quantitation and cDNA Synthesis

Fourth instar larvae (whole body) were collected and stored at -82ºC. Five larvae were collected from each of the five replications of control, and the 138.24 µg/ml SWCNT treatment and the 4.32 µg/ml and 8.64 µg/ml AgNP treatment arenas. For total RNA extraction, the larvae were placed in liquid nitrogen and crushed using a porcelain mortar and pestle. All the equipment's were cleaned with 70% ethanol and RNase-Away (Molecular BioProducts Inc., San Diego, CA, USA) to eliminate contamination. The

larvae were then homogenized in 750 µl Trizol Reagent (life technologies, Carlsbad, CA, USA). Total RNA was precipitated with 500 µl isopropanol and resuspended in 50 µl DEPC treated water.

Extracted RNA was purified with Qiagen RNeasy® Mini Kit (Thermo Fisher Scientific, Eugene, Oregon, USA) following manufacturer's guidelines. I used Qubit® 3.0 Fluorometer and Qubit® RNA BR assay kits (Invitrogen, Eugene, Oregon, USA) to quantitate the RNA following the manufacturer's guidelines. The samples were then stored at -80ºC until next use.

Three samples containing RNA at high concentration were selected from each treatment and first strand cDNA was synthesized with Qiagen QuantiTect® reverse transcription kit (Chatsworth, CA, USA) following the manufacturer's guidelines. I also performed reverse transcription using both oligo dT and random primers, but the QuantiTect® kit provided the highest yield of cDNA. All RNA samples were reverse transcribed simultaneously to avoid variations in cDNA. The cDNA was then amplified by regular PCR and confirmed with agarose gel electrophoresis. cDNA was stored at -20ºC until next use.

2.8. Quantitative Real-Time PCR (qRT-PCR) Analysis

Permethrin resistance gene CYP6BG,1 a member of the p450 gene family, was used as a target gene, and ribosomal protein L32 (RPL32) was used as a reference gene for qRT-PCR. The primer design for CYP6BG1 was reported previously by Bautista *et al*. (2007). Fu *et al.* (2013) and Gao *et al*. (2016) reported that RPL 32 is a reliable

housekeeping gene. I used the Integrated DNA Technologies website to design the forward and reverse primer for RPL 32 (Table 2).

The qPCR was performed with GoTaq® qPCR master mix (Promega Corporation, Madison, WI, USA) in an Mx3000P thermal cycler (Agilent Technologies, Inc. Santa Clara, CA, USA) with the help of MxPro qPCR software. 96 well EU thin-walled PCR plates (BPCTi inc., Durham, NC, USA) were used for the reaction. To determine PCR amplification efficiency, standard curves were generated for both target and reference gene using 10-fold serial dilutions. Thermal cycling profiles used in this study were: 95ºC for 10 min, followed by 40 cycles of 95ºC for 30 sec, 55ºC for 60 sec, 72ºC for 60 sec. A dissociation step cycle at 95ºC for 60 sec, 50ºC for 30 sec and 95ºC for 30 sec was added as a final step to generated melting curves. The amplification reaction was done in three technical replicates for each biological replicate. No-template controls (NTC) were run for every sample to check for DNA contamination. The gene expression level was calculated based on cycle threshold (C_t) value by using Pfaffl method (Pfaffl *et al*., 2002).

2.9. Statistical analysis

Data were analyzed using statistical software Minitab 17 (State College, PA; USA). I used a general linear model ANOVA to test for treatment effects on feed consumption, larval growth, % pupation, % survival, fecundity, and gene expression level. Data are presented as mean ± standard error of mean. Tukey's test was performed for pairwise comparisons when main treatment effects in the ANOVA were statistically significant at $p<0.05$. I started feeding trials using 8.64 µg/ml SWCNT in artificial feed (Trial 1). In every subsequent trial, I doubled the concentration until I reached a concentration of 138.24 µg/ml SWCNT, the highest concentration that I could

incorporate into the artificial feed. I used untransformed, natural log-transformed and Box-Cox transformed data because consumption and growth data are generally not normally distributed. However, there was no significant differences in the results. Consequently, I present untransformed data.

RESULTS

3.1. Effect of SWCNTs on DBM

3.1.1. Effect of SWCNTs on Feed Consumption. There was no evidence that any concentration of SWCNT deterred feeding (Table 3). If the CNT deterred feeding than consumption of CNT feed would have always been less then consumption of control feed. There were no clear negative or positive effects of CNTs on consumption (Table 3). Larval feeding was not consistently affected by any concentration. For example, consumption of feed containing 8.64 µg/ml SWCNT was not significantly different than control (Appendix parts 1 and 2); consumption of 17.28 µg/ml SWCNT feed was not significantly different from the consumption of control feed (Appendix parts 1 and 2). However, 69.12 µg/ml and 138.24 µg/ml feed larvae consumed 10% and 4% more food than control food. Taken together, while there were some concentration and trials that suggested an apparent SWCNTs affects, there was no clear significant negative or positive effect detected (Appendix parts 1-6).

3.1.2. Effect of SWCNTs on growth. SWCNTs did not significantly affect DBM growth rate, except at the concentration of 138.24 µg/ml (Table 4 and Appendix parts 1-6). For example, 8.64 µg/ml and 17.28 µg/ml feed larvae growth rate was same and lower (Table 4) compared to control, which cancelled each other's out. 34.56 µg/ml feed larvae growth rate was same compared to control. But, 69.12 µg/ml and 138.24 µg/ml feed larvae growth rate was reduced almost 6% and 10% respectively. Since the larva became darkened in color (Figure 1) the SWCNTs may have not been metabolized but accumulated in their gut.

3.1.3. Effect of SWCNTs on the rate of pupation. SWCNTs did not had any measurable negative effect on the percentage of DBM pupation (Table 5). For the lowest SWCNT concentration of 8.64 µg/ml, there was 100% pupation. However, there was a decreasing pupation rate with increasing SWCNT concentrations. For example: 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml, and 138.24 µg/ml SWCNT fed larvae showed 3%, 4%, 6% and 4% reduction of pupation rate, respectively (Table 5). Even though the results were not significant, this reduced pupation rate suggests that there may be some level of SWCNT toxicity to DBMs.

3.1.4. Effect of SWCNT on the rate of survival. There was no significant effect of SWCNT on DBM survival rate (Table 6). For lower SWCNT concentrations i.e. 8.64 µg/ml and 17.28 µg/ml the survival was 100%. However, with the increasing SWCNT concentrations the survival rate decreased (Table 6). Survival rate reduced 2% for 34.56 µg/ml, 6% for 69.12 µg/ml and 3% for 138.24 µg/ml SWCNT treatment. Even though the reduced survival rate was not significant, the observed trend warrants further investigation.

3.1.5. Effect of SWCNT on feed conversion. As the SWCNT fed larvae growth rate was same or less with same or higher consumption rate compared to control, we further investigated their feed conversion ratio; percentage of µg growth for µg feed consumption for each larva (Table 7). For example, 8.64 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml and138.24 µg/ml SWCNT feed larvae converted 10%, 3%, 6%, 16% and 12% less compared to control (Appendices 8-13).

3.1.6. Effect of SWCNT on fecundity. I investigated potential SWCNT effect on their fecundity for trial 4,5 and 6 (Table 8). All the SWCNT fed larvae showed reduced

fecundity relative to control fed larvae. Fecundity was reduced by almost 17% and 45% with elevated SWCNT concentration (69.12 µg/ml and 138.24 µg/ml, respectively) which is an indication of a sub-lethal toxic effect.

3.1.7. Effect of SWCNTs Across All Data. I combined and analyzed the entire data set for all the variables (Table 9). Consumption of feed containing SWCNTs was not significantly different than consumption of control feed. Growth was statistically significantly affected, even though the growth rate had an increasing tendency from 8.64 µg/ml to 69.12 µg/ml, at highest concentration (138.24 µg/ml) the growth rate decreased. For 50 hours, the larvae grew at a similar rate, after which their growth rate ended up at around same point (Appendices 22-27). Their pupation rate was not significantly different for individual trials. But the accumulated pupation rate is significantly different. Except for the lowest concentration (8.64 µg/ml), For all the SWCNTs concentrations (17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml, and 138.24 µg/ml), DBM showed negatively correlated with reduced pupation rates, except for 8.64 µg/ml SWCNT. I also examined survival rate, which was not statistically affected by SWCNTs. Fecundity (egg/adult) was negatively correlated statistically significantly affected, with the increasing with SWCNT concentration, fecundity was with an overall reduction of reduced by 45% in fecundity. This negative correlation was significant. Conversion rate was statistically significant, lower concentrations has lower conversion ratio compared to control, only our highest concentration 138.24 µg/ml has higher conversion ratio than control. This might be insect's antioxidant defense response (Lee *et al*., 2015)

The regression line for each SWCNT concentration showed that larva fed with 8.64 µg/ml of SWCNT feed larva grew more as they consumed more feed. ate more, but

control larvae the growth rate of the control larvae was stable over time (Figure 2.a). Larvae fed with 17.28 µg/ml of SWCNT feed larval growth did not increase with increasing amount of feeding (Figure 2.b), whereas, the control feed larvae grew less with increased feeing. Control larvae and larvae fed with 34.56 µg/ml of SWCNT and control feed larvae had a reduced growth rate was reduced with increasing feeding (Figure 2.c). Larvae fed with 69.12 µg/ml and 138.28 µg/ml of SWCNT feed larvae grew parallelly with control feed larvae, they ate less and grew less (Figure 2.d and Figure 2.e).

3.2. Effect of AgNP on DBM

There were two artificial feed assays to investigate the direct effect of AgNPs (4.32 µg/ml and 8.64 µg/ml) on DBM. I investigated the effect of AgNPs on consumption, growth, survival rate, pupation rate, and fecundity. AgNPs negatively affected DBM consumption (Table 10). At the level of both 4.32 µg/ml and 8.64 µg/ml of AgNP, larvae ate less AgNP feed than control. DBM showed reduced growth rate for both AgNPs concentration, but the reduction was not significant (Table 11). However, demelanized and stunted larvae were observed during artificial feed assays (Figure 3). AgNP reduced pupation rate by 10% for the 8.64 µg/ml of AgNPs treatment (Table 12). I could not measure an impact on survival rate (Table 13) at either concentration of AgNPs used. But in my experiments, I tested very low AgNP concentration. Conversion rate differences were not significant (Table 14). For both AgNP concentration (4.32 µg/ml and 8.64 µg/ml), conversion ratio was both higher and lower (Appendix parts 19 and 18 respectively) compared to control. For AgNPs, fecundity was not statistically significant

(Table 15). In trial 1, both AgNP concentration increased fecundity, where as in trial 2, the fecundity remains almost the same.

For the combined analysis, the consumption and pupation rate were significantly reduced on AgNP-fed larvae (Table 16). Consumption reduced significantly for both concentrations, but pupation rate was significantly reduced for the 8.64 µg/ml treatment only. This suggests sublethal toxicity of AgNPs to DBM. Growth, survival rate, fecundity, and conversion rate remained the same with control treatment (Table 16). From the regression lines, there was no clear relationship for AgNP-feeding and growth (Figure 4).

3.3. Effect of ENPs on CYP6BG1 gene expression level

I performed qRT-PCR, and calculated relative gene expression levels using the Pfaffl method to detect differences in the expression of the CYP6BG1 gene expression between treated and control DBM. The efficiency for reference and target gene was 97.5% and 83.8% (Appendix parts 28 and 29) respectively. The expression level of CYP6BG1 gene was higher for all the ENP treatments (Figure 5), but they were not significantly different from control DBM (Appendix 21).

DISCUSSION

4.1 Effect of SWCNT on DBM

There was no dose dependent toxic effect of SWCNTs on DBM feeding. Fraser *et al.* (2011) also did not find any significant dose dependent feeding effect of SWCNTs on rainbow trout, even though they used very high concentration (500 mg/kg) and different sized SWCNTs (outside diameter = 1.1 nm, length = 5–30 mm). To my knowledge, my research is the first to examine feeding by a caterpillar on artificial feed containing various concentrations of nanomaterials. As the consumption rate of larvae fed on 69.12 µg/ml and 138.24 µg/ml SWCNT was higher than control, I am speculating that, the larvae didn't receive the same rate of nutrients from the SWCNT food compared to control and started compensatory feeding. Probably C molecule inhibiting the larvae from metabolizing the SWCNT food.

I did not find any significant effect of SWNTS on DBM growth rate except at the highest concentration (138.24 µg/ml). My results are consistent with the findings of: Philbrook *et al*. (2011), who reported no effect of SWCNT treatment on maternal weight of pregnant mice for 10 mg/kg SWCNT with >90% purity and functionalized hydroxyl group. Fraser *et al*. (2011) found no effect of 500 mg/kg SWCNT on rainbow trout growth rate; Cheng *et al*. (2007) reported same body length of zebrafish for 240 mg/L SWCNT treatment; Templeton *et al*. (2006) reported same growth rate of copepods for purified or as prepared 10 mg/L SWCNT; and Leeuw *et al*. (2007) reported same growth rate of fruit fly for ∼10 ppm SWCNT.

Several researchers have reported that SWCNTs can accumulate in organs and the body cavity of animals as diverse as insects and mammals. For example, Holt *et al*.

(2010) reported incorporation of SWCNTs into centromere structure of actin filaments; Kang *et al*. (2007) reported SWCNT aggregation into bacterial cells; Mwangi *et al*. (2012) found accumulated SWCNT into amphipod, midge, and oligochaete gut; Leeuw *et al.* (2007) found accumulated SWCNT on drosophila tissue; Petersen *et al*. (2008) reported accumulated SWCNT into *Lumbriculus variegatus* gut; Roberts *et al*. (2007) reported deposited SWCNT into daphnia gut. Even though SWCNTs may not be acutely toxic and may not hinder growth of animals, except at very high concentration, they are being transferred through the feed chain (Roberts *et al*., 2007). SWCNTs can be chronically toxic to animals even in low concentrations (Smith *et al*., 2007). They are suspected to be more toxic to mammals than insects, which suggests that there can be biomagnification.

I found that for the higher SWCNT concentrations I examined (34.56 µg/ml, 69.12 µg/ml, and 138.24 µg/ml) pupation rate decreased, albeit the effect was not significant. One possibility is that this is due to sample size. Experimental report from Lee *et al*. (2015) on fruit fly for CNF supports my result, even though they tested larger particle-size (100 nm diameter) and higher concentration (1000 µg/ml) of CNF compared to the one used here.

I found that only the highest concentration of SWCNT may have any impact on survival, although it was not possible to measure this effect in a significant manner. There are many previous studies that found similar results. For example: Philbrook *et al*. (2011), Fraser *et al*. (2011), Cheng *et al*. (2007), Templeton *et al*. (2006), Leeuw *et al*. (2007), Liu *et al*. (2009), Lee *et al*. (2015) and Shon *et al*. (2014). Some studies also reported decreased survival rate and reduced viability. For example, Kisin *et al*. (2007)

reported reduced survival rate in human lung fibroblast cell line; Mwangi *et al*. (2012) reported reduced survival rate of amphipod, midge, and oligochaete; and Templeton *et al*. (2006) found reduce viability of copepods. All these studies used very diverse SWCNTs (different size and concentration), which makes it impossible to infer any overarching conclusion from these studies.

As mentioned in the introduction, animals are probably not able to metabolize SWCNTs. However, the possibility of chronic toxicity exists, and future research examining predators eating DMB feed with or without SWCNTs is warranted. Also, the feeding trials lasted for about 60-70 hours: only in 3rd and 4th instars. It is possible that this exposure is too short and that these later two instar stages have higher resistance than previous stages.

In my experiments, I found that fecundity was reduced by almost 45% at the highest SWCNT concentration, which is an indication of sub- lethal toxic effects. There are several published reports, i.e. Cheng *et al*. (2007), Templeton *et al*. (2006) and Lee *et al*. (2015) which supports my findings.

From all the combined analysis, I can say that SWCNTs have sub-lethal toxicity on DBM growth, pupation rate and fecundity. Further experiments on SWCNTs chronic toxicity on DBM are warranted.

4.2. Effect of AgNPs on DBM

AgNPs are deterrent to DBM feeding, even at lowest tested concentration of 4.32 µg/ml. There are few studies on AgNPs feeding rate. There are numerous studies on AgNPs negative effect on growth of various animals: Kim *et al*., (2010) reported reduced

body weight in rats for 500 mg/Kg AgNP; Bar-Ilan *et al*., (2009) reported stunted growth on zebrafish for 250 µM AgNP; and Zhao and Wang, (2011) reported reduced growth rate of *daphnia* for 5 µg/ml AgNP. All these experiments tested higher AgNP concentration than I tested in my experiments. Furthermore, I observed deformed, demelanized larvae with stunted growth, which is also similar with other experiments. For example, Mathias *et al*. (2014), Bar-Ilan *et al*. (2009), Lee *et al*. (2007b), Armstrong *et al*. (2013), Silver-Key *et al*. (2011) and Posgai *et al*. (2011).

I found reduced pupation rate for 8.64 µg/ml AgNP concentration. Posgai *et al*. (2011) also reported similar results but their AgNP concentration was much higher than mine. I did not find any AgNP effect on DBM's survival rate. Armstrong *et al*. (2013) also reported no significant effect of adult survival on *drosophila* for 50 mg/L; Zhao and Wang (2011), reported no mortality of *daphnia* for 500 µg/ml. There was no significant effect of AgNP on DBM's fecundity. Silver Key *et al*. (2011) reported unaffected hatching rates were on *drosophila* for 5%/ ~9000 ppm.

4.3. ENPs effect on CYP6BG1 gene expression level

I found that CYP6BG1 gene expression was higher in DMB feed ENPs than those feeding on feed with no ENPs. Even though my results were statistically significant, further studies are warranted. Bautista *et al*. (2007, 2009) reported and confirmed upregulation of CYP6BG1 gene due to permethrin resistance in DBM. CYP P450 gene family have 36 to 180 genes in insect genome (Feyereisen, 2012; Zhou *et al*., 2014), which makes it very hard to target one or few genes for gene expression assessment due to ENPs exposure.

In conclusion, my study provides new insights on the effect of functionalized pure (>95%) SWCNT on intact arthropods, relevant to ecotoxicity studies and environmental concerns. In my study, I did not find any acute toxicity as determined by measuring consumption, growth, % pupation and % survival rate. But I cannot exclude the subtle and sublethal toxic effects at different trials. My study provides strong suggestion for further study of SWCNT effect on insect fecundity. Also, SWCNT accumulation in insect body cavity should be further investigated for their biochemical and genomic toxicity.

There are many studies on AgNPs toxicity to animals and at higher concentration and they were identified as toxic in most cases. Even though, I tested very lower concentration, they showed toxicity for consumption and % pupation. Future studies with higher concentration is needed to verify their toxicity on arthropods.

My study on CYP6BG1 gene expression emphasize that, insects specially DBM have very strong immunity and CYP P450 family helps insects to metabolize these toxic ENPs. De novo expression profile analysis due to ENPs exposure to DBM would be very interesting study.

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Table 1: The concentrations of single walled carbon nanotube (SWCNT) and AgNPs tested and the number of replicates for each feeding trial.

ENP	Trial	Treatments	No. of replicates
SWCNT	1	0 µg/ml, 8.64 µg/ml, 17.28 µg/ml, NLC, NFC	4x
	2	0 µg/ml, 8.64 µg/ml, 17.28 µg/ml, NLC, NFC	4x
	3	0 µg/ml, 17.28 µg/ml, 34.56 µg/ml, NLC, NFC	8x
	4	0 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml, NLC, NFC	5x
	5	0 µg/ml, 34.56 µg/ml, 69.12 µg/ml, 138.24 µg/ml, NLC, NFC	5x
	6	0 µg/ml, 138.24 µg/ml, NLC, NFC	5x
AgNP	1	0 µg/ml, 4.32 µg/ml, 8.64 µg/ml, NLC, NFC	5x
	2	0 µg/ml, 4.32 µg/ml, 8.64 µg/ml, NLC, NFC	5x

Table 2: Primer design and properties used for qRT-PCR

CYP6BG1		Amplicon length	T_m	GC content
Forward	ACCCTCGAGAAGGGTCTCCGA	111	61.7	61.9
Reverse	ATTCTCCGGCGAAAACCGATC		57.7	52.4
RPL32				
Forward	CAATTTACCGCCCTACCATC	91	53.4	50
Reverse	CGCCAGTTACGCTTTATTTTG		52.7	42.9

Table 3: Mean (±SE) consumption by diamondback moths (DMB) of feed without and with increasing concentrations of SWCNTs across six trials. Different letters (a- c) represent significant differences (* for p≤0.05, ** for P ≤ 0.01 and *** for P ≤ 0.001) among different SWCNT concentration (0 µg/ml, 8.64 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml, and 138.24 µg/ml).

Trial	Concentration of SWCNT in artificial feed					
	0 µg/ml	8.64 µg/ml	17.28 µg/ml	34.56 µg/ml	69.12 µg/ml	138.24 µg/ml
1	1432.45 ±53.0	1408.23 ±40.3	1466.73 ±75.1			
2	1577.01 ±40.1	1596.24 ±11.1	1564.01 ±15.5			
3*	1718.83 ±25.4[a]		1847.16 ±41.5[b]	1802.92 ±12.2[a, b]		
4***	1408.42 ±13.9[a]		1336.4 1±12.2[b]	1307.74 ±16.8[b]	1407.75 ±9.3[a]	
5***	1214.90 ±12.6[a]			1430.51 ±44.9[b, c]	1530.31 ±63.9[c]	1296.69 ±19.9[a, b]
6	1462.22 ±69.7					1486.83 ±137

Table 4: Mean (±SE) growth of diamondback moths (DMB) feeding on artificial feed without and with increasing concentrations of SWCNTs. Different letters (a- b) represent significant differences (* for $p \leq 0.05$) among different SWCNT concentration (0 µg/ml, 8.64 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml, and 138.24 µg/ml).

Trial	Concentration of SWCNT in artificial feed					
	0 µg/ml	8.64 µg/ml	17.28 µg/ml	34.56 µg/ml	69.12 µg/ml	138.24 µg/ml
1*	96.94 ±3.6[a]	77.05 ±6.3[b]	79.79 ±2.3[a, b]			
2	94.08 ±4.9	95.90 ±5.9	93.30 ±6.1			
3	95.30 ±4.9		100.74 ±4.7	95.92 ±4.2		
4	107.60 ±3.1		110.88 ±1.8	104.88 ±3.0	110.48 ±2.8	
5	108.77 ±3.9			104.84 ±4.5	93.65 ±6.5	99.07 ±3.2
6*	110.67 ±3.0[a]					99.90 ±2.0[b]

Table 5: Mean (±SE) % of pupation of diamondback moth (DBM) across six different trials for different SWCNT concentration (0 µg/ml, 8.64 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml, and 138.24 µg/ml).

Trial	Concentration of SWCNT in artificial feed					
	0 µg/ml	8.64 µg/ml	17.28 µg/ml	34.56 µg/ml	69.12 µg/ml	138.24 µg/ml
1	96.67 ±1.92	100.00 ±0.00	95.00 ±1.67			
2	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00			
3	99.17 ±0.83		95.00 ±2.44	96.67 ±2.52		
4	100.00 ±0.00		94.67 ±3.89	97.33 ±1.63	96.00 ±1.63	
5	100.00 ±0.00			96.00 ±1.63	92.00 ±4.90	96.00 ±1.63
6	100.00 ±0.00					96.00 ±2.45

Table 6: Mean (±SE) % of survival of DBM across six different trials for different SWCNT concentration (0 µg/ml, 8.64 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml, and 138.24 µg/ml).

Trial	Concentration of SWCNT in artificial feed					
	0 µg/ml	8.64 µg/ml	17.28 µg/ml	34.56 µg/ml	69.12 µg/ml	138.24 µg/ml
1	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00			
2	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00			
3	100.00 ±0.00		100.00 ±0.00	100.00 ±0.00		
4	89.42 ±3.42		86.38 ±4.82	86.38 ±3.61	92.86 ±3.91	
5	100.00 ±0.00			97.24 ±1.96	90.94 ±4.31	98.57 ±1.43
6	96.00 ±2.45					91.78 ±2.07

Table 7: Percentage of µg growth (±SE) per µg consumption for Diamondback Moth across six different trials. Different letters (a- c) represent significant differences (* for p≤0.05, ** for P ≤ 0.01 and *** for P ≤ 0.001) among different SWCNT concentration (0 µg/ml, 8.64 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml, and 138.24 µg/ml).

Trial	Concentration of SWCNT in artificial feed					
	0 µg/ml	8.64 µg/ml	17.28 µg/ml	34.56 µg/ml	69.12 µg/ml	138.24 µg/ml
1*	6.77 ±0.1[a]	5.47 ±0.4[b]	5.47 ±0.2[b]			
2	5.99 ±0.4	6.01 ±0.4	5.97 ±0.4			
3	5.54 ±0.3		5.47 ±0.3	5.3 3±0.2		
4	7.64 ±0.2		8.30 ±0.1	8.02 ±0.2	7.85 ±0.2	
5 ***	8.95 ±0.3[a]			7.38 ±0.5[b, c]	6.12 ±0.3[c]	7.65 ±0.3[a, b]
6	7.64 ±0.4					6.92 ±0.6

Table 8: Number of eggs per adult (±SE) for Diamondback Moth across three different trials. Different letters (a-b) represent significant differences (* for p≤0.05) among different SWCNT concentration (0 µg/ml, 8.64 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml, and 138.24 µg/ml).

Trial	Concentration of SWCNT in artificial feed					
	0 µg/ml	8.64 µg/ml	7.28 µg/ml	34.56 µg/ml	69.12 µg/ml	138.24 µg/ml
4	11.9±4.0		5.7±2.4	13.0±2.8	15.1±6.5	
5*	27.4±4.4[a]			24.9±3.9[a, b]	17.6±5.6[a, b]	9.2±2.9[b]
6	19.7±5.7					10.9±3.6

Table 9: Mean (±SE) data for different variables of Diamondback Moth across six different trials. Different letters (a- c) represent significant differences (* for p≤0.05, ** for P ≤ 0.01 and *** for P ≤ 0.001) among different SWCNT concentration (0 µg/ml, 8.64 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml, and 138.24 µg/ml). In case of egg/adult and conversion, its statistically significant, but Tukey t-test at 95% confidence interval did not show any grouping (Appendices 7 and 14).

Variables	Concentration of SWCNT in artificial feed					
	0 µg/ml	8.64 µg/ml	17.28 µg/ml	34.56 µg/ml	69.12 µg/ml	138.24 µg/ml
Consumption	1490.84 ± 33.9	1502.23 ±40.5	1599.16 ±50.8	1561.92 ± 55.1	1469.03 ±36.7	1391.76 ±72.6
Growth*	101.99 ±2.1[a]	86.47 ±5.3[b]	97.74 ±3.1[a, b]	100.89 ±2.5[a, b]	102.06 ±4.4[a, b]	99.48 ±1.8[a, b]
% Pupation**	99.36 ±0.4[a]	100 ±0.0[a, b]	95.87 ±1.3[a, b]	96.67 ±1.2[a, b]	94.00 ±2.5[b]	96.00 ±1.4[a, b]
% survival	97.65 ±9.4	100 ±0.0	96.76 ±1.7	95.45 ±1.7	91.90 ±2.8	95.17 ±1.6
Egg/ Adult*	19.66 ±3.0		5.7 ±2.4	18.94 ±3.0	16.36 ±4.1	10.07 ±2.2
Conversion*	6.99 ±0.2	5.74 ±0.3	6.24 ±0.3	6.49 ±0.3	6.98 ±0.3	7.29 ±0.3

Table 10: Mean (±SE) consumption rate of AgNPs on DBM across two different trials. Different letters (a- c) represent significant differences (* for p ≤ 0.05, ** for P ≤ 0.01 and *** for P ≤ 0.001) among different AgNPs concentration (0 µg/ml, 4.32 µg/ml and 8.64 µg/ml).

Trial	Concentration of AgNP in artificial feed		
	0 µg/ml	4.32 µg/ml	8.64 µg/ml
1*	1462.22±69.7[a]	1309.96±61.3[a, b]	1182.54±27.3[b]
2***	1617.5±24.9[a]	1317.6±20.6[c]	1456.0±22.8[b]

Table 11: Mean (±SE) growth rate of Diamondback Moth across two different trials among different AgNPs concentration (0 µg/ml, 4.32 µg/ml and 8.64 µg/ml).

Trial	Concentration of AgNP in artificial feed		
	0 µg/ml	4.32 µg/ml	8.64 µg/ml
1	110.67±2.95	92.90±9.61	87.12±8.13
2	81.41±5.83	68.62±5.60	75.44±5.75

Table 12: Mean (±SE) pupation (%) rate of Diamondback Moth across two different trials. Different letters (a- b) represent significant differences (** for p≤0.01) among different AgNPs concentration (0 µg/ml, 4.32 µg/ml and 8.64 µg/ml).

Trial	Concentration of AgNP in artificial feed		
	0 µg/ml	4.32 µg/ml	8.64 µg/ml
1**	100.00±0.00[a]	100.00±0.00[a]	90.00±3.16[b]
2	100±0.00	98.00±2.00	98.00±2.00

Table 13: Mean (±SE) survival (%) rate of Diamondback Moth across two different trials. Different letters (a- b) represent significant differences (** for p≤0.01) among different AgNPs concentration (0 µg/ml, 4.32 µg/ml and 8.64 µg/ml).

Trial	Concentration of AgNP in artificial feed		
	0 µg/ml	4.32 µg/ml	8.64 µg/ml
1	96.00±2.45	100.00±0.00	95.28±2.90
2	100±0.00	94.00±4.00	100.00±0.00

Table 14: Percentage of µg growth per µg consumption for Diamondback Moth across two different trials among different AgNPs concentration (0 µg/ml, 4.32 µg/ml and 8.64 µg/ml).

Trial	Concentration of AgNP in artificial feed		
	0 µg/ml	4.32 µg/ml	8.64 µg/ml
1	7.64±0.42	7.10±0.71	7.32±0.54
2	5.02±0.31	5.23±0.48	5.18±0.38

Table 15: Number of eggs per adult for DBM across three different trials among different AgNPs concentration (0 µg/ml, 4.32 µg/ml and 8.64 µg/ml).

Trial	Concentration of AgNP in artificial feed		
	0 µg/ml	4.32 µg/ml	8.64 µg/ml
1	19.74±5.69	40.20±10.1	33.42±10.4
2	29.52±7.65	28.91±3.70	29.32±5.00

Table 16: Mean (±SE) data for different variables of Diamondback Moth across two different trials. Different letters (a- b) represent significant differences (* for p≤0.05 and ** for P ≤ 0.01) among different AgNPs concentration (0 µg/ml, 4.32 µg/ml and 8.64 µg/ml).

Variables	Concentration of AgNP in artificial feed		
	0 µg/ml	4.32 µg/ml	8.64 µg/ml
Consumption**	1539.9± 43.5[a]	1313.8±30.5[b]	1319.3±48.6[b]
Growth	96.04±5.7	80.76±6.62	81.28±5.08
%Pupation*	100.00±0.0[a]	99.00±1.0[a]	94.00±2.2[b]
% Survival	98.00±1.3	97.00±2.13	97.64±1.6
Egg/Adult	24.63±4.8	34.55±5.4	31.37±5.5
Conversion	6.33±0.50	6.16±0.51	6.25±0.47

1.a. Control feed

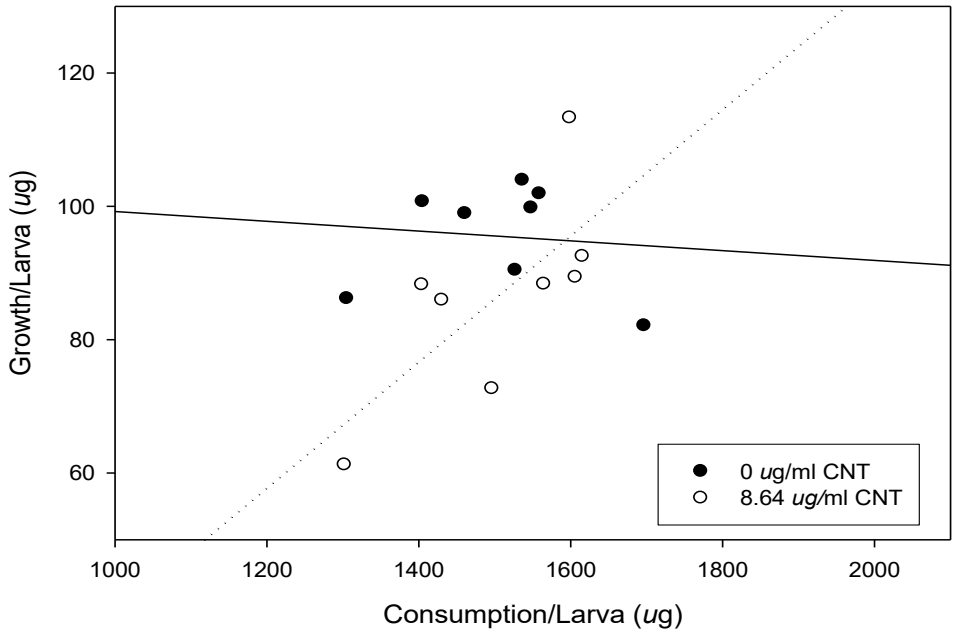
1.c. SWCNT feed

1.b. Control feed larva

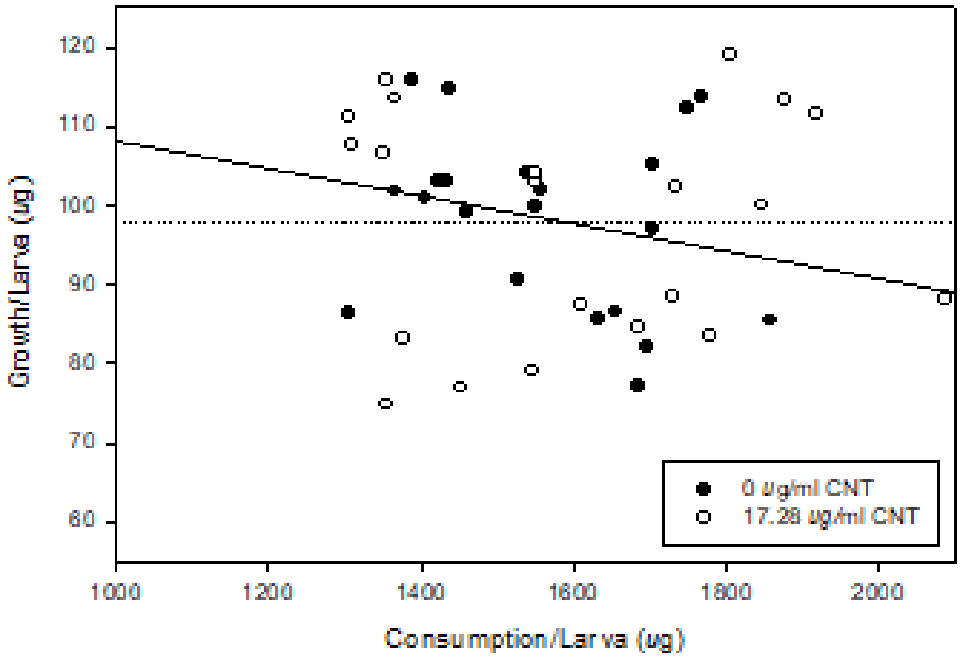
1.d. SWCNT feed larva

Figure 1: DBM larvae exposed to artificial feed. (1.a.) Control feed with larvae. (1.c.) SWCNT feed at higher concentration (138.24 µg/ml) with larvae. The SWCNT feed are very much darker than control feed. (1.b.) Larvae feeding on control feed, clear gut. (1.d.) Larvae feed on SWCNT feed, accumulated SWCNT on their gut. Evidence that SWCNT were taken with feed and accumulated in their body.

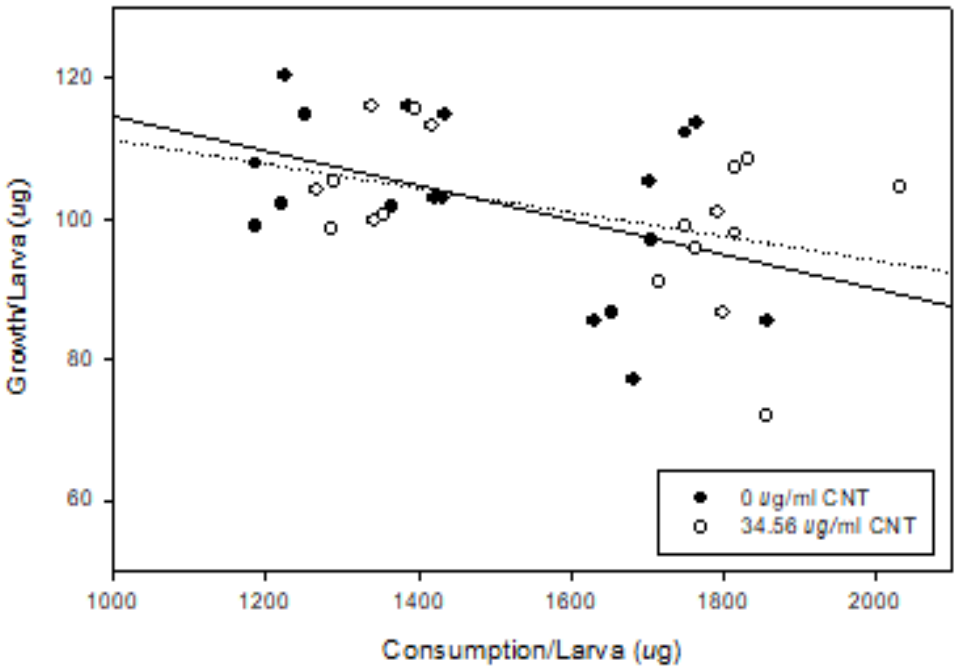
a


120
100
80
60
Growth/Larva (ug)
1000
1200
1400
1600
1800
2000
Consumption/Larva (ug)
0 ug/ml CNT
8.64 ug/ml CNT

b


120
110
100
90
80
70
60
Growth/Larva (ug)
1000
1200
1400
1600
1800
2000
Consumption/Larva (ug)
0 ug/ml CNT
17.28 ug/ml CNT

c


120
100
80
60
Growth/Larva (ug)
1000
1200
1400
1600
1800
2000
Consumption/Larva (ug)
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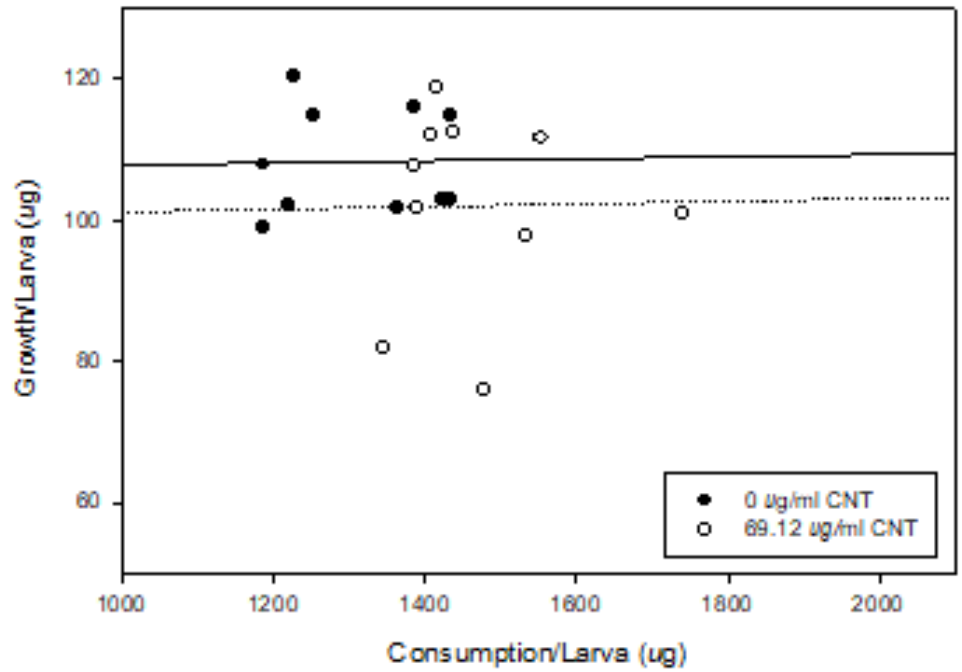
34.56 ug/ml CNT

d


120
100
80
60
Growth/Larva (ug)
1000
1200
1400
1600
1800
2000
Consumption/Larva (ug)
0 ug/ml CNT
69.12 ug/ml CNT

e

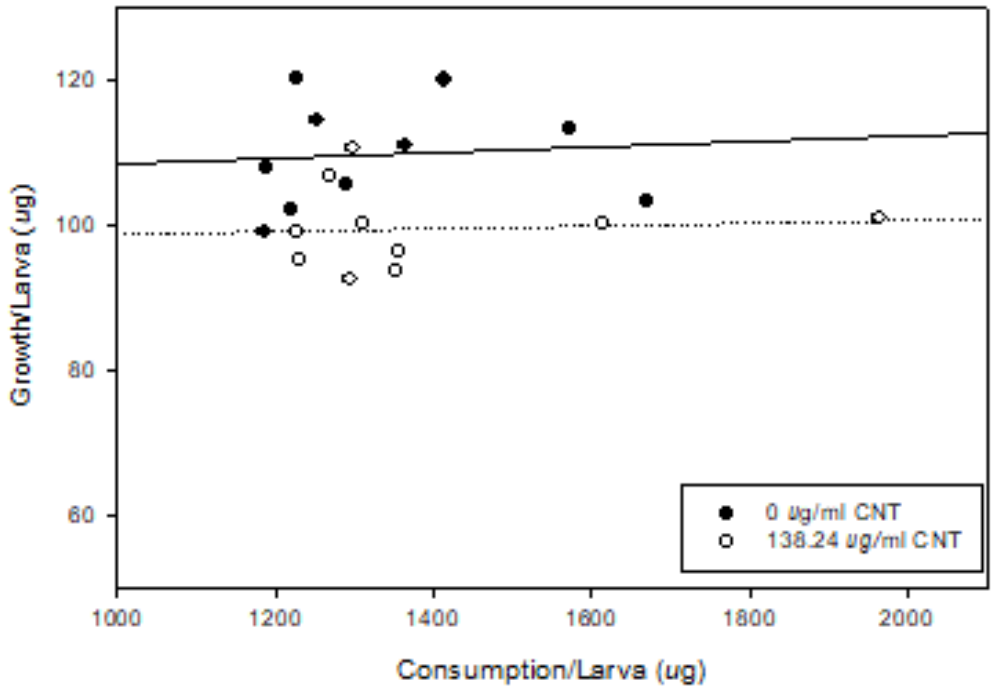
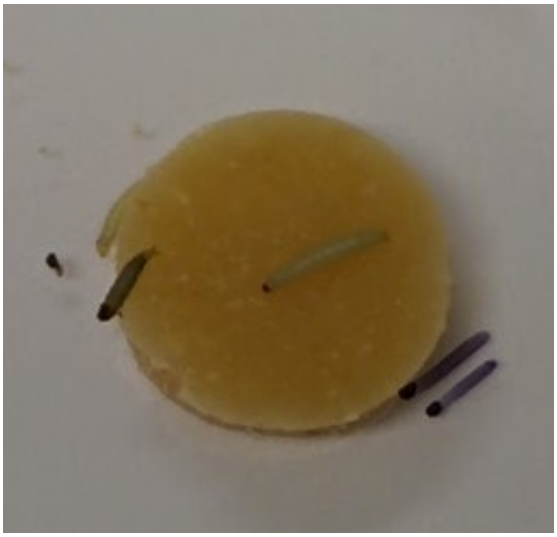


Figure 2. Scatter plot of growth/larva (µg) for consumption/larva (µg) of diamondback moth exposed to different SWCNT concentration. The average of controls for different SWCNT concentrations are different, as there were several trials for different concentrations. a) 0 µg/ml and 8.64 µg/ml. b) 0 µg/ml and 17.28 µg/ml. c) 0 µg/ml and 34.56 µg/ml. d) 0 µg/ml and 69.12 µg/ml). e) 0 µg/ml and 138.24 µg/ml. Solid line denotes the regression for 0 µg/ml and dotted line denotes the regression for SWCNT concentration.

3.a. Control feed

3.c. AgNP feed

3.b. Control feed larva



3.d. AgNP feed larva

Figure 3: DBM larvae exposed to artificial feed. (3.a.) Control feed with larvae. (3.c.) AgNP feed at higher concentration (8.64 µg/ml) with larvae. The AgNP feed are a little brownish than control feed. (3.b.) Larvae feeding on control feed, healthy and normal length. (3.d.) Larvae feed on AgNP feed, a dying larva with shorter length.

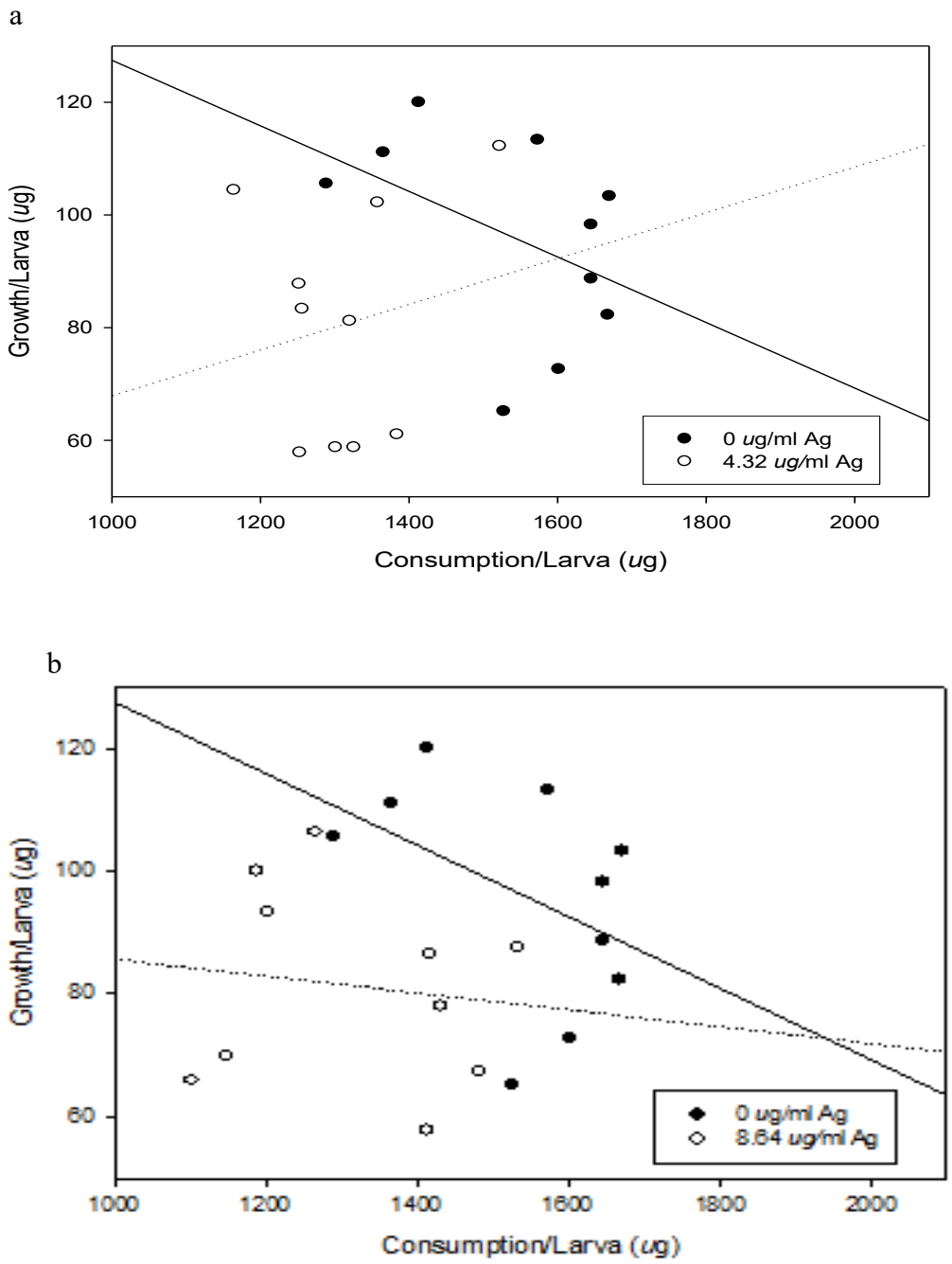


Figure 4. Scatter plot of growth/larva (µg) for consumption/larva (µg) of diamondback moth exposed to different AgNPs concentration, a) 0 µg/ml and 4.32 µg/ml, b) 0 µg/ml and 8.64 µg/ml. Solid lines denotes the regression for 0 µg/ml and dotted lines denotes the regression for AgNPs.

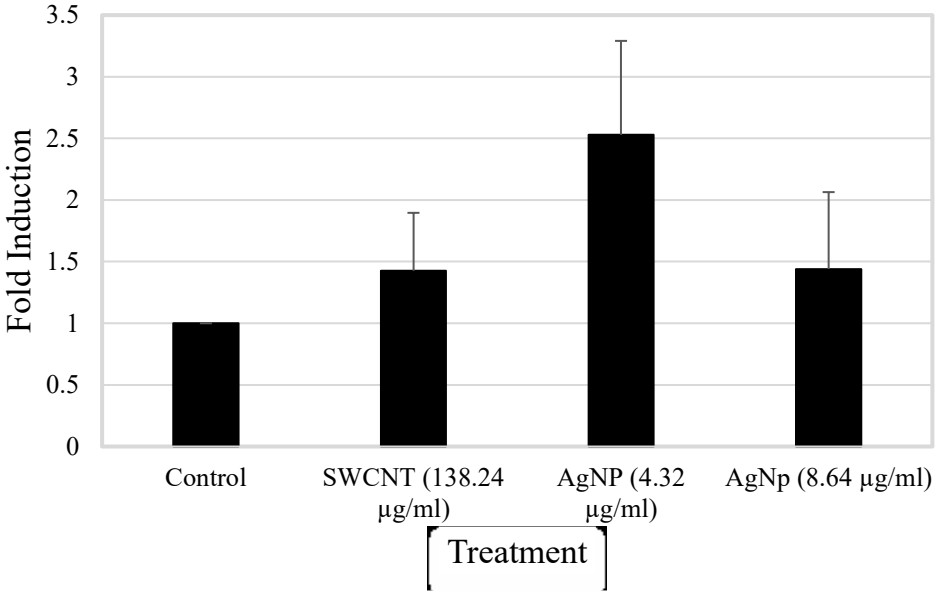


Figure 5: CYP6BG1 gene expression level in DMB feeding on different concentrations and types of ENPs

APPENDIX

1: Mean (±SE) consumption, growth, pupation, and survival of DBMs feeding on artificial food without or with SWCNTs (8.64 µg/ml and 17.28 µg/ml) from feeding trial 1. For this trial, consumption (df = 2; F = 0.26 and p = 0.779), pupation (df = 2; F = 03.00 and p = 0.100) and survival was not significantly affected by the presence of SWCNTs in the food. Growth (df = 2; F = 6.00 and p = 0.022) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H (µg)	0 µg/ml SWCNT	1432.45	53.00
	8.64 µg/ml SWCNT	1408.23	40.30
	17.28 µg/ml SWCNT	1466.73	75.10
Growth/L/H (µg) *	0 µg/ml SWCNT	96.94[a]	3.60
	8.64 µg/ml SWCNT	77.05[b]	6.30
	17.28 µg/ml SWCNT	79.79[a, b]	2.30
% Pupation	0 µg/ml SWCNT	96.67	1.92
	8.64 µg/ml SWCNT	100	0
	17.28 µg/ml SWCNT	95	1.67
% Survival	0 µg/ml SWCNT	100	0
	8.64 µg/ml SWCNT	100	0
	17.28 µg/ml SWCNT	100	0

2: Mean (±SE) consumption, growth, pupation, and survival of DBM feeding on artificial food without or with SWCNT (8.64 µg/ml and 17.28 µg/ml) from feeding trial 2. For this trial, consumption (df = 2; F = 0.4 and p = 0.681), growth (df = 2; F = 0.06 and p = 0.946), pupation and survival was not significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H (µg)	0 µg/ml SWCNT	1577.01	40.10
	8.64 µg/ml SWCNT	1596.24	11.10
	17.28 µg/ml SWCNT	1564.01	15.50
Growth/L/H (µg)	0 µg/ml SWCNT	94.08	4.90
	8.64 µg/ml SWCNT	95.90	5.90
	17.28 µg/ml SWCNT	93.30	6.10
% Pupation	0 µg/ml SWCNT	100	0
	8.64 µg/ml SWCNT	100	0
	17.28 µg/ml SWCNT	100	0
% Survival	0 µg/ml SWCNT	100	0
	8.64 µg/ml SWCNT	100	0
	17.28 µg/ml SWCNT	100	0

3: Mean (±SE) consumption, growth, pupation, and survival of DBM feeding on artificial food without or with SWCNTs (17.28 µg/ml and 34.56 µg/ml) from feeding trial 3. For this trial, growth (df = 2; F = 0.42 and p = 0.661), pupation (df = 2; F = 1.02 and p = 0.379) and survival was not significantly affected by the presence of SWCNTs in the food. Consumption (df = 2; F = 5.08 and p = 0.016) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H (µg) *	0 µg/ml SWCNT	1718.83[a]	25.40
	17.28 µg/ml SWCNT	1847.16[b]	41.50
	34.56 µg/ml SWCNT	1802.92[a, b]	12.20
Growth/L/H (µg)	0 µg/ml SWCNT	95.30	4.90
	17.28 µg/ml SWCNT	100.74	4.70
	34.56 µg/ml SWCNT	95.92	4.20
% Pupation	0 µg/ml SWCNT	99.17	0.83
	8.64 µg/ml SWCNT	95.00	2.44
	17.28 µg/ml SWCNT	96.67	2.52
% Survival	0 µg/ml SWCNT	100	0
	17.28 µg/ml SWCNT	100	0
	34.56 µg/ml SWCNT	100	0

4: Mean (±SE) consumption, growth, pupation, survival and egg/adult of DBM feeding on artificial food without or with SWCNTs (17.28 µg/ml, 34.56 µg/ml, and 69.12 µg/ml) from feeding trial 4. For this trial, growth (df = 3; F = 1.06 and p = 0.393), pupation (df = 2; F = 1.01 and p = 0.412), survival (df = 2; F = 0.60 and p = 0.624, and egg/adult (df = 3; F = 0.91 and p = 0.457) was not significantly affected by the presence of SWCNTs in the food. Consumption (df = 3; F = 14.65 and p ≤ 0.0001) was highly significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H (µg) ***	0 µg/ml SWCNT	1408.42[a]	13.90
	17.28 µg/ml SWCNT	1336.41[b]	12.20
	34.56 µg/ml SWCNT	1307.74[b]	16.80
	69.12 µg/ml SWCNT	1407.75[a]	9.30
Growth/L/H (µg)	0 µg/ml SWCNT	107.60	3.10
	17.28 µg/ml SWCNT	110.88	1.80
	34.56 µg/ml SWCNT	104.88	3.00
	69.12 µg/ml SWCNT	110.48	2.80
% Pupation	0 µg/ml SWCNT	100	0
	17.28 µg/ml SWCNT	94.67	3.89
	34.56 µg/ml SWCNT	97.33	1.63
	69.12 µg/ml SWCNT	96.00	1.63
% Survival	0 µg/ml SWCNT	89.42	3.42
	17.28 µg/ml SWCNT	86.38	4.82
	34.56 µg/ml SWCNT	86.38	3.61
	69.12 µg/ml SWCNT	92.86	3.91
Egg/Adult	0 µg/ml SWCNT	11.9	4.00
	17.28 µg/ml SWCNT	5.7	2.40
	34.56 µg/ml SWCNT	13.0	2.80
	69.12 µg/ml SWCNT	15.1	6.50

5: Mean (±SE) consumption, growth, pupation, survival and egg/adult of DBMS feeding on artificial food without or with SWCNTs (34.56 µg/ml, 69.12 µg/ml and 138.24 µg/ml) from feeding trial 5. For this trial, growth (df = 3; F = 1.99 and p = 0.156), pupation (df = 3; F = 1.45 and p = 0.264) and survival (df = 3; F = 2.73 and p = 0.079) was not significantly affected by the presence of SWCNTs in the food. Consumption (df = 3; F = 11.77 and p ≤ 0.0001) and egg/adult (df = 3; F = 3.63 and p = 0.036) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H (µg) ***	0 µg/ml SWCNT	1214.90[a]	12.60
	34.56 µg/ml SWCNT	1430.51[b, c]	44.90
	69.12 µg/ml SWCNT	1530.31[c]	63.90
	138.24 µg/ml SWCNT	1296.69[a, b]	19.90
Growth/L/H (µg)	0 µg/ml SWCNT	108.77	3.90
	34.56 µg/ml SWCNT	104.84	4.50
	69.12 µg/ml SWCNT	93.65	6.50
	138.24 µg/ml SWCNT	99.07	3.20
% Pupation	0 µg/ml SWCNT	100	0
	34.56 µg/ml SWCNT	96.0	1.63
	69.12 µg/ml SWCNT	92.0	4.90
	138.24 µg/ml SWCNT	96.0	1.63
% Survival	0 µg/ml SWCNT	100	0
	34.56 µg/ml SWCNT	97.24	1.96
	69.12 µg/ml SWCNT	90.94	4.31
	138.24 µg/ml SWCNT	98.57	1.43
Egg/Adult*	0 µg/ml SWCNT	27.4[a]	4.40
	34.56 µg/ml SWCNT	24.9[a, b]	3.90
	69.12 µg/ml SWCNT	17.6[a, b]	5.60
	138.24 µg/ml SWCNT	9.2[b]	2.90

6: Mean (±SE) consumption, growth, pupation, and survival of DBM feeding on artificial food without or with SWCNTs (138.24 µg/ml) from feeding trial 6. For this trial, consumption (df = 1; F = 0.3 and p 0.877), pupation (df = 1; F = 2.67 and p = 0.141), survival (df = 1; F = 1.74 and p = 0.224) and egg/adult (df = 1; F = 1.69 and p = 0.230) was not significantly affected by the presence of SWCNTs in the food. Growth (df = 1; F = 9.1 and p = 0.017) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H (µg)	0 µg/ml SWCNT	1462.22	69.70
	138.24 µg/ml SWCNT	1486.83	137.00
Growth/L/H (µg) *	0 µg/ml SWCNT	110.67[a]	3.00
	138.24 µg/ml SWCNT	99.90[b]	2.00
% Pupation	0 µg/ml SWCNT	100.0	0
	138.24 µg/ml SWCNT	96.0	2.45
% Survival	0 µg/ml SWCNT	96.0	2.45
	138.24 µg/ml SWCNT	91.78	2.07
Egg/Adult	0 µg/ml SWCNT	19.7	5.70
	138.24 µg/ml SWCNT	10.9	3.60

7: Mean (±SE) consumption, growth, pupation, survival and egg/adult of DBM feeding on artificial food without or with SWCNTs (8.64 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml and 138.24 µg/ml) from all combined feeding trial. For this trial, consumption (df = 5; F = 1.86 and p = 0.109) and survival (df = 5; F = 1.87 and p = 0.108) was not significantly affected by the presence of SWCNTs in the food. Growth (df = 5; F = 2.33 and p = 0.049), pupation (df = 5; F = 3.22 and p = 0.01) and egg/adult were statistically significant (df = 4; F = 2.72 and p = 0.041) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H (µg)	0 µg/ml SWCNT	1490.84	33.90
	8.64 µg/ml SWCNT	1502.23	40.50
	17.28 µg/ml SWCNT	1599.16	50.80
	34.56 µg/ml SWCNT	1561.92	55.10
	69.12 µg/ml SWCNT	1469.03	36.70
	138.24 µg/ml SWCNT	1391.76	72.60
Growth/L/H (µg) *	0 µg/ml SWCNT	101.99[a]	2.10
	8.64 µg/ml SWCNT	86.47[b]	5.30
	17.28 µg/ml SWCNT	97.74[a, b]	3.10
	34.56 µg/ml SWCNT	100.89[a, b]	2.50
	69.12 µg/ml SWCNT	102.06[a, b]	4.40
	138.24 µg/ml SWCNT	99.48[a, b]	1.80
% pupation*	0 µg/ml SWCNT	99.36[a]	0.40
	8.64 µg/ml SWCNT	100.00[a, b]	0.00
	17.28 µg/ml SWCNT	95.87[a, b]	1.30
	34.56 µg/ml SWCNT	96.67[a, b]	1.20
	69.12 µg/ml SWCNT	94.00[b]	2.50
	138.24 µg/ml SWCNT	96.00[a, b]	1.40
% survival	0 µg/ml SWCNT	97.65	0.94
	8.64 µg/ml SWCNT	100.0	0
	17.28 µg/ml SWCNT	96.76	1.67
	34.56 µg/ml SWCNT	95.45	1.73
	69.12 µg/ml SWCNT	91.90	2.76
	138.24 µg/ml SWCNT	95.17	1.64
Egg/Adult*	0 µg/ml SWCNT	19.7	3.00
	17.28 µg/ml SWCNT	5.7	2.40
	34.56 µg/ml SWCNT	18.9	3.00
	69.12 µg/ml SWCNT	16.4	4.10
	138.24 µg/ml SWCNT	10.1	2.20

8: Mean (±SE) conversion ratio (percentage of µg growth for µg food consumption for each larva) of DBM feeding on artificial food without or with SWCNTs (8.64 µg/ml and 17.28 µg/ml) from feeding trial 1. For this trial, conversion (df = 2; F = 7.53 and p = 0.012) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion*	0 µg/ml SWCNT	6.771[a]	0.142
	8.64 µg/ml SWCNT	5.465[b]	0.40
	17.28 µg/ml SWCNT	5.466[b]	0.22

9: Mean (±SE) conversion ratio (percentage of µg growth for µg food consumption for each larva) of DBMs feeding on artificial food without or with SWCNTs (8.64 µg/ml and 17.28 µg/ml) from feeding trial 2. For this trial, conversion (df = 2; F = 0.00 and p = 0.998) wasn't significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion	0 µg/ml SWCNT	5.994	0.42
	8.64 µg/ml SWCNT	6.007	0.36
	17.28 µg/ml SWCNT	5.970	0.41

10: Mean (±SE) conversion ratio (percentage of µg growth for µg food consumption for each larva) of DBMs feeding on artificial food without or with SWCNTs (17.28 µg/ml and 34.56 µg/ml) from feeding trial 3. For this trial, conversion (df = 2; F = 0.18 and p = 0.835) wasn't significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion	0 µg/ml SWCNT	5.544	0.27
	17.28 µg/ml SWCNT	5.471	0.27
	34.56 µg/ml SWCNT	5.326	0.24

11: Mean (±SE) conversion ratio (percentage of µg growth for µg food consumption for each larva) of DBMs feeding on artificial food without or with SWCNTs (17.28 µg/ml, 34.56 µg/ml and 69.12 µg/ml) from feeding trial 4. For this trial, conversion (df = 3; F = 2.2 and p = 0.128) wasn't significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion	0 µg/ml SWCNT	7.642	0.27
	17.28 µg/ml SWCNT	8.297	0.12
	34.56 µg/ml SWCNT	8.022	0.22
	69.12 µg/ml SWCNT	7.846	0.17

12: Mean (±SE) conversion ratio (percentage of µg growth for µg food consumption for each larva) of DBMs feeding on artificial food without or with SWCNTs (34.56 µg/ml, 69.12 µg/ml and 138.24 µg/ml) from feeding trial 5. For this trial, conversion ($df = 3$; $F = 11.29$ and $p \leq 0.0001$) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion***	0 µg/ml SWCNT	8.948[a]	0.27
	34.56 µg/ml SWCNT	7.376[b, c]	0.45
	69.12 µg/ml SWCNT	6.117[c]	0.38
	138.24 µg/ml SWCNT	7.651[a, b]	0.29

13: Mean (±SE) conversion ratio (percentage of µg growth for µg food consumption for each larva) of DBMs feeding on artificial food without or with SWCNTs (138.24 µg/ml) from feeding trial 6. For this trial, conversion ($df = 1$; $F = 1.02$ and $p = 0.342$) wasn't significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion	0 µg/ml SWCNT	7.642	0.42
	138.24 µg/ml SWCNT	6.920	0.58

14: Mean (±SE) conversion ratio (percentage of µg growth for µg food consumption for each larva) of DBMS feeding on artificial food without or with SWCNTs (8.64 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml and 138.24 µg/ml) from all combined feeding trial. Conversion (df = 5; F = 2.33 and p = 0.049) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion*	0 µg/ml SWCNT	6.986	0.25
	8.64 µg/ml SWCNT	5.736	0.27
	17.28 µg/ml SWCNT	6.238	0.29
	34.56 µg/ml SWCNT	6.492	0.34
	69.12 µg/ml SWCNT	6.982	0.34
	138.24 µg/ml SWCNT	7.285	0.33

15: Mean (±SE) consumption, growth, pupation, and survival of DBMs feeding on artificial food without or with AgNP (4.32 µg/ml and 8.64 µg/ml) from feeding trial 1. For this trial, growth (df = 2; F = 2.70 and p = 0.107), survival (df = 2; F = 1.35 and p = 0.297) and egg/adult (df = 2; F = 1.34 and p = 0.299) wasn't significantly affected by the presence of AgNPs in the food. Consumption (df = 2; F = 6.28 and p = 0.014) and pupation (df = 2; F = 10.00 and p = 0.003) was significantly affected by the presence of AgNPs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different AgNP concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H (µg) *	0 µg/ml AgNP	1462.22[a]	69.70
	4.32 µg/ml AgNP	1309.96[a, b]	61.30
	8.64 µg/ml AgNP	1182.54[b]	27.30
Growth/L/H (µg)	0 µg/ml AgNP	110.67	2.95
	4.32 µg/ml AgNP	92.90	9.61
	8.64 µg/ml AgNP	87.12	8.13
% pupation**	0 µg/ml AgNP	100.00[a]	0.00
	4.32 µg/ml AgNP	100.00[a]	0.00
	8.64 µg/ml AgNP	90.00[b]	3.16
% Survival	0 µg/ml AgNP	98.00	1.33
	4.32 µg/ml AgNP	97.00	2.13
	8.64 µg/ml AgNP	97.64	1.58
Egg/Adult	0 µg/ml AgNP	19.74	5.69
	4.32 µg/ml AgNP	40.20	10.10
	8.64 µg/ml AgNP	33.43	10.40

16: Mean (±SE) consumption, growth, pupation, and survival of DBMs feeding on artificial food without or with AgNP (4.32 µg/ml and 8.64 µg/ml) from feeding trial 2. For this trial, growth (df = 2; F = 1.25 and p = 0.321), pupation (df = 2; F = 0.50 and p = 0.619), survival (df = 2; F = 2.25 and p = 0.148) and egg/adult (df = 2; F = 0.00 and p = 0.997) was not significantly affected by the presence of AgNPs in the food. Consumption (df = 2; F = 43.16 and p ≤ 0.0001) was significantly affected by the presence of AgNPs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different AgNP concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H (µg) *	0 µg/ml AgNP	1617.5[a]	24.9
	4.32 µg/ml AgNP	1317.6[c]	20.6
	8.64 µg/ml AgNP	1456.0[b]	22.8
Growth/L/H (µg)	0 µg/ml AgNP	81.41	5.83
	4.32 µg/ml AgNP	68.62	5.60
	8.64 µg/ml AgNP	75.44	5.75
% pupation	0 µg/ml AgNP	100.00	0.00
	4.32 µg/ml AgNP	98.00	2.00
	8.64 µg/ml AgNP	98.00	2.00
% survival	0 µg/ml AgNP	96.0	2.45
	4.32 µg/ml AgNP	100.0	0.0
	8.64 µg/ml AgNP	95.28	2.90
Egg/Adult	0 µg/ml AgNP	29.52	7.65
	4.32 µg/ml AgNP	28.91	3.70
	8.64 µg/ml AgNP	29.32	5.00

17: Mean (±SE) consumption, growth, pupation, and survival of DBMs feeding on artificial food without or with AgNP (4.32 µg/ml and 8.64 µg/ml) from all combined feeding trial. Growth (df = 2; F = 2.19 and p = 0.131), % survival (df = 2; F = 0.09 and p = 0.917) and egg/adult (df = 2; F = 0.93 and p = 0.405) was not significantly affected by the presence of AgNPs in the food. Consumption (df = 2; F = 9.64 and p = 0.001) and pupation (df = 2; F = 5.26 and p = 0.012) was significantly affected by the presence of AgNPs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different AgNP concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H (µg) **	0 µg/ml AgNP	1539.9[a]	43.50
	4.32 µg/ml AgNP	1313.8 [b]	30.50
	8.64 µg/ml AgNP	1319.3[b]	48.60
Growth/L/H (µg)	0 µg/ml AgNP	96.04	5.77
	4.32 µg/ml AgNP	80.76	6.62
	8.64 µg/ml AgNP	81.28	5.08
% pupation*	0 µg/ml AgNP	100.00[a]	0.00
	4.32 µg/ml AgNP	99.00[a]	1.00
	8.64 µg/ml AgNP	94.00[b]	2.21
% survival	0 µg/ml AgNP	100.0	0.0
	4.32 µg/ml AgNP	94.0	4.0
	8.64 µg/ml AgNP	100.0	0.0
Egg/Adult	0 µg/ml AgNP	24.63	4.78
	4.32 µg/ml AgNP	34.56	5.43
	8.64 µg/ml AgNP	31.37	5.50

18: Mean (±SE) conversion ratio (percentage of µg growth for µg food consumption for each larva) of DBMs feeding on artificial food without or with AgNP (4.32 µg/ml and 8.64 µg/ml) from feeding trial 1. For this trial, conversion (df = 2; F = 0.23 and p = 0.798) wasn't significantly affected by the presence of AgNPs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different AgNP concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion	0 µg/ml AgNP	7.64	0.42
	4.32 µg/ml AgNP	7.10	0.71
	8.64 µg/ml AgNP	7.32	0.54

19: Mean (±SE) conversion ratio (percentage of µg growth for µg food consumption for each larva) of DBMs feeding on artificial food without or with AgNPs (4.32 µg/ml and 8.64 µg/ml) from feeding trial 2. For this trial, conversion (df = 2; F = 0.08 and p = 0.927) wasn't significantly affected by the presence of AgNPs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different AgNP concentrations as determined using Tukey's test.

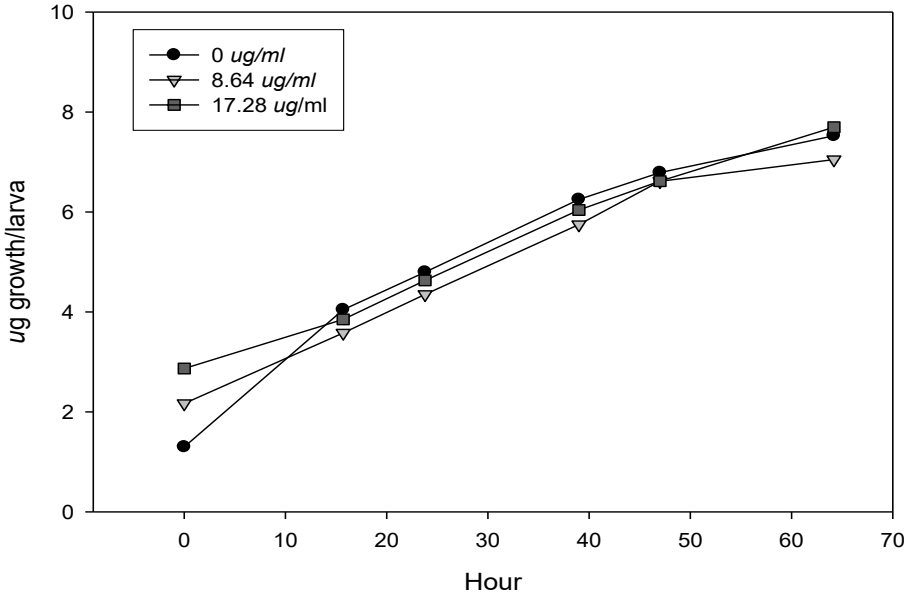
Variable	Treatment	Mean	SE Mean
Conversion	0 µg/ml AgNP	5.02	0.31
	4.32 µg/ml AgNP	5.23	0.48
	8.64 µg/ml AgNP	5.18	0.38

20: Mean (±SE) conversion ratio (percentage of µg growth for µg food consumption for each larva) of DBMs feeding on artificial food without or with AgNP (4.32 µg/ml and 8.64 µg/ml) from all combined feeding trial. Conversion (df = 2; F = 0.03 and p = 0.972) wasn't significantly affected by the presence of AgNPs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different AgNP concentrations as determined using Tukey's test.

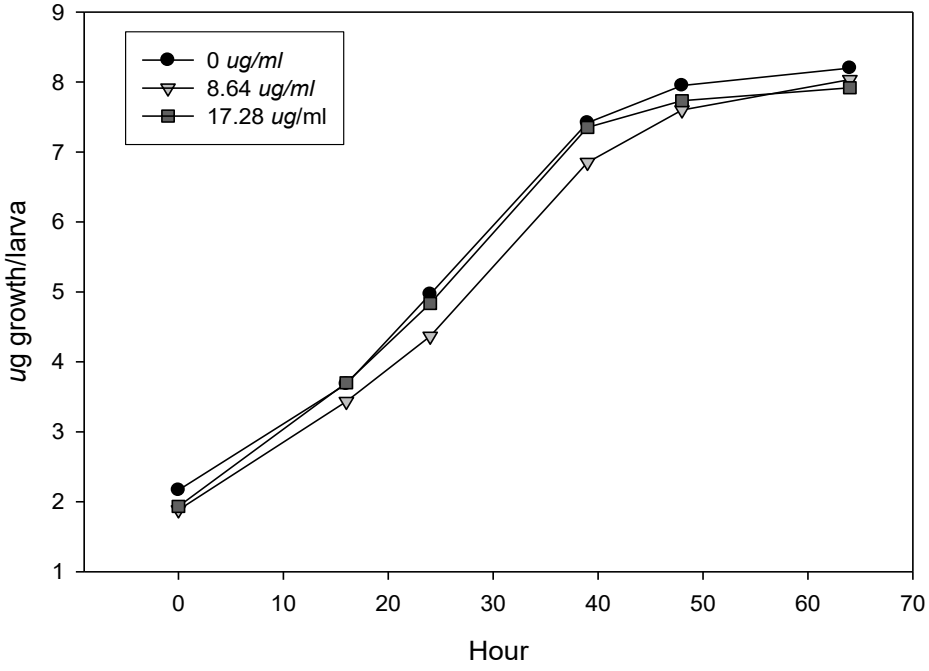
Variable	Treatment	Mean	SE Mean
Conversion	0 µg/ml AgNP	6.33	0.50
	4.32 µg/ml AgNP	6.16	0.51
	8.64 µg/ml AgNP	6.25	0.47

21: Mean (±SE) gene expression of CYP6BG1 of DBMs feeding on artificial food without or with SWCNT (138.24 µg/ml) and AgNP (4.32 µg/ml and 8.64 µg/ml) from feeding trial 6 and 1. Gene expression (df = 3; F = 1.43 and p = 0.252) wasn't significantly affected by the presence of SWCNT and AgNP in the food.

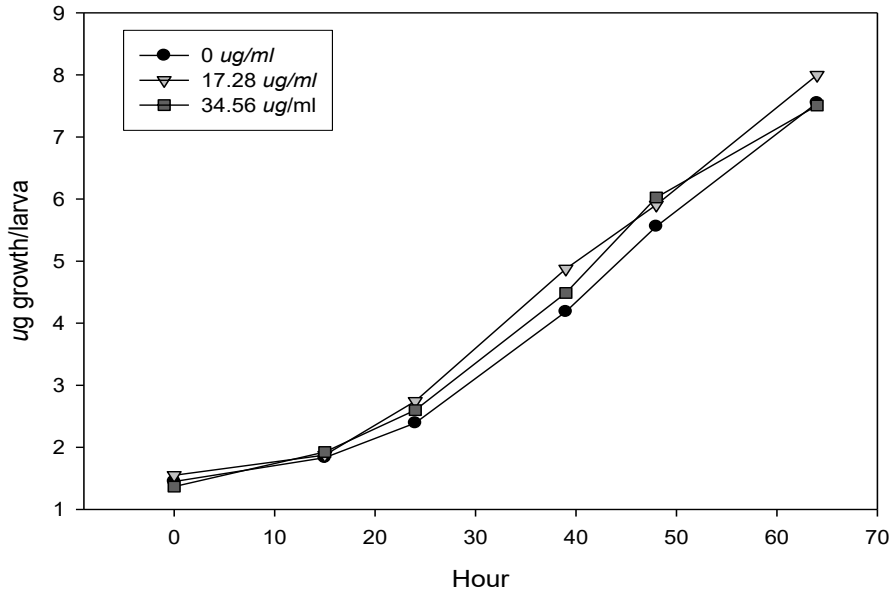
Variable	Treatment	Mean	SE Mean
Fold change of gene	0 µg/ml	1.0	0.0
	138.24 µg/ml SWCNT	1.43	0.47
	4.32 µg/ml AgNP	2.53	0.76
	8.64 µg/ml AgNP	1.44	0.62



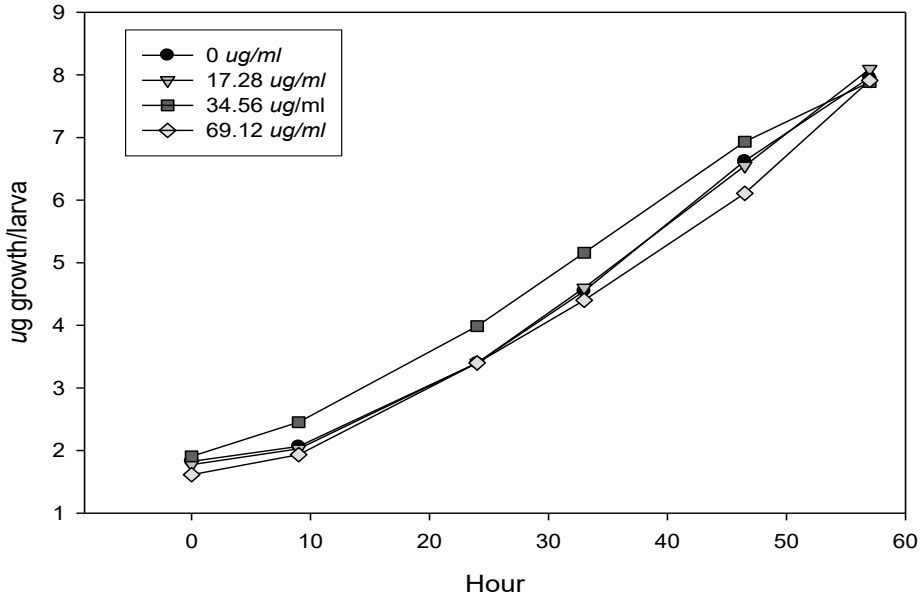
22: Larval growth (µg) along with time for trial 1 for control and different SWCNT concentration food.



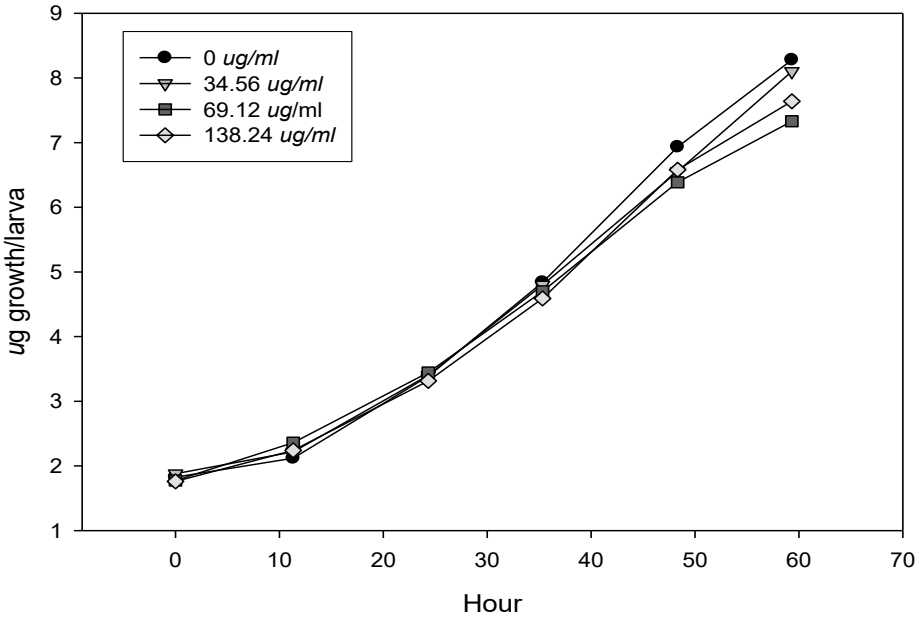
23: Larval growth (µg) along with time for trial 2 for control and different SWCNT concentration food



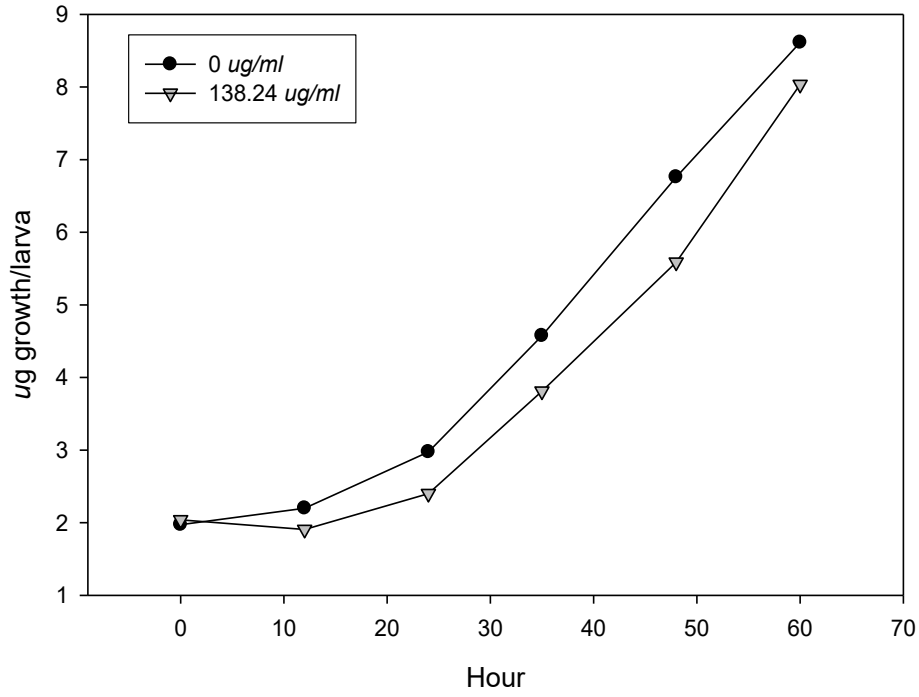
24: Larval growth (µg) along with time for trial 3 for control and different SWCNT concentration food



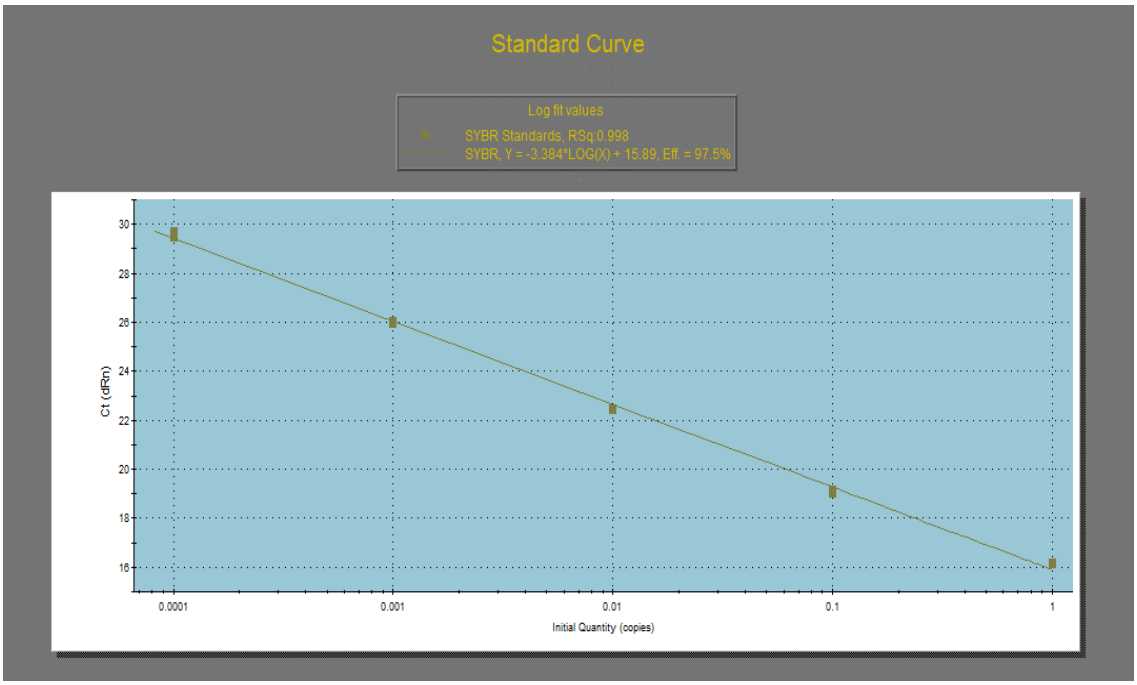
25: Larval growth (µg) along with time for trial 4 for control and different SWCNT concentration food



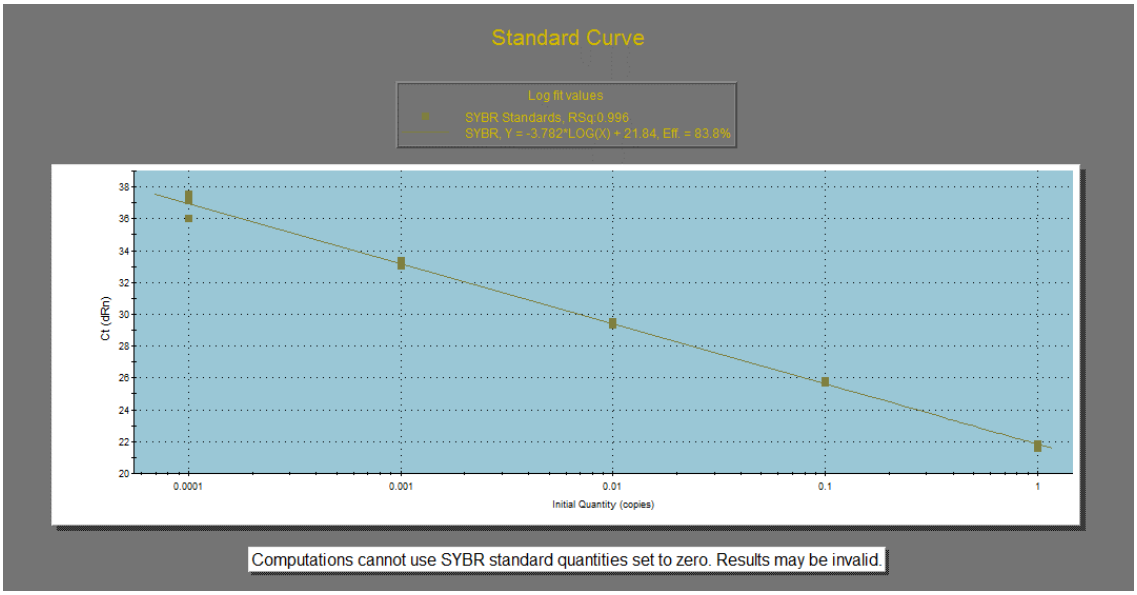
26: Larval growth (µg) along with time for trial 5 for control and different SWCNT concentration food



27: Larval growth (µg) along with time for trial 6 for control and different SWCNT concentration food



28: Standard curve for reference gene RPL 32



29: Standard curve for target gene CYP6BG1