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Toxicity Study of the Traditional and Nano-form of Propamocarb- -HCl on Cucumber and their Toxicity on HepG2 Line

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PHYTOTOXIC effects of propamocarb-HCl pesticide and its Nano form on cucumber plant and their adverse impact on tissue culture of HepG2 line were studied. Nano form was loaded on chitosan nanoparticles (NPs). Significant increase in plant morphological parameters was noticed after the last periods of transplanting. Also, it exhibited significant increases in fruits yield greater than the traditional form. The quality recognized the same pattern. All treatments: Doses, ½ doses, and double doses showed an increase in leave pigments in the case of nano-form treatments greater than the traditional form. Biosafety profile on HepG2 cell lines showed a slight difference with 1.22 folds between the traditional and nano-form. While, lactate dehydrogenase (LDH) and malondialdehyde (MDA) showed a significant increase, compared to their control. Such a finding provides the advantage of nano-formation on vegetable crops, but it must be coupled with further toxicological studies.

Keywords: Propamocarb- HCl; Cucumber; Nanoparticles; Phytotoxicity; *In vitro* toxicity.

1. Introduction

Phytotoxic denotes injury or death to plants. The level of a chemical or other compound's toxicity towards plants is known as its phytotoxicity. The phytotoxic effects of some pesticides may be presented in negative effects on the growth rate of the treated plant. Its effects might range from mild leaf browning or scorching to plant death. Affected leaves, fruit, flowers, and stems might occasionally appear deformed (Hajjar et al. 2014). However, extensive use of pesticides has caused contamination of products and the environment in most areas (Liu et al. 2018). Furthermore, more than 90% of pesticides leach into the environment during application due to flaws in typical pesticide formulations such as the use of hazardous solvents, poor dispersion, dust drift, and so on. (Hassaan and El Nemr 2020).

In recent years, the use of nanotechnology enables the development of new formulations that have the potential to increase the efficacy and safety of pesticides. Formulations based on nanotechnology are being developed for pesticides, using controlled release mechanisms to precisely release the necessary and suitable amounts of its active ingredient (a. i) in response to environmental stimulation and biological requirements (Zhao et al. 2017). Pesticides can benefit from nanotechnology by having lower toxicity, improved shelf life, and higher solubility of poorly water-soluble pesticides, all of which can have a favorable environmental

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impact (Neme et al. 2021). Nanoparticles (NPs) can be utilized to manage plant diseases in two ways: as NPs in and of themselves or as nano-carriers for pesticides and RNA interference agents. Despite the numerous potential benefits of using NPs, several NP-based products have yet to be marketed for agriculture (Worrall et al. 2018). The systemic fungicide propamocarb-HCl is used to control phytophthora diseases of important crops. (Hu et al., 2007). It is widely used as a fungicide around the world (Abd-Alrahman and Almaz 2012). Every year, plant pests and pathogens caused 20 to 40% of crop productions are lost (Anderson et al., 2004). Pseudoperonospora-caused cucumber downy mildew is a dangerous leaf disease that can spread quickly and reduce cucumber yields. Consequently, Cucumber quality and yield were impacted, which then resulted in financial losses. One of the most commonly used substances worldwide is pesticides. Up to 80% of the crops have been protected from pests and weeds, depending on how they are used in contemporary agriculture (Awais, 2019; Uebbing et al (2023). There are many previous studies on soil pollution such as vehicular emission pollutants (Sarhan et al. 2021), micro-plastic (Elbasiouny et al. 2022), industrial pollutants (Hussein et al. 2022), heavy metal pollutants (Abd-El-Hady and Abdelaty 2022), nano-pollutants (Faizan et al. 2023). Whereas, as far as we know, few publications on the nano-forms of propamocarb-HCl pollutants and their toxicity on cultivated cucumber can be found.

The objective of the current study is to assess the phytotoxic effects of propamocarb-HCl and its nano-form on cucumber plants under greenhouse conditions. Also, evaluate their impact on the human cell line.

2. Material and Methods

2.1. Chemicals and reagents

Fungicide propamocarb-HCl (Arakure® 72% SC) was obtained from Egypt Agricultural Development Co., Egypt). Propamocarb-HCl is a carbamate is applied as a bare-root dip (soaking) before transplanting. The doses were prepared in the distilled water independent in recommended rate as follows: (250 cm^3/100 L water), 2- times dose (500 cm^3/100 L water) and half dose (125 cm^3/100 L water).

2.2 Preparation and characterization of Chitosan-propamocarb-HCl nanoparticles

The preparation of chitosan-propamocarb NPs according to (Calvo et al. 1997) with some modifications. By dissolving chitosan (CS) in an acetic acid solution (1% v/v) at room temperature, an aqueous solution (0.2% w/v) of CS was obtained. Following that, a cross-linking agent solution of sodium tripolyphosphate (TPP) (0.06% w/v) was added as drops to the CS solution with continuous stirring for 30 minutes. The NPs were prepared by adding chitosan solution to propamocarb- HCl by probe sonicator (UP400ST, Hielscher, Germany) for 5 min at 50% amplitude in an ice bath to avoid overheating in presence of Tween 80 as a surfactant to reduce NPs hydrodynamic diameter. The morphology of the produced chitosan-propamocarb NPs was imaged using a High Resolution-Transmission Electron Microscope (HR-TEM) set to 200 Kv (Tecnai G2, FEI, Netherlands). For five minutes, an ultrasonicated solution of diluted chitosan-propamocarb NPs was used. On a carbon-coated copper grid, three drops of the sonicated solution were applied, and they were then allowed to dry at room temperature. For morphological assessment, HR-TEM photos of the NPs were taken. The mean particle size distribution found by the zeta sizer (Malvern, ZS Nano, UK) was estimated using the dynamic light scattering (DLS) approach. X-ray Diffraction (XRD) analysis was used to determine the chemical structure of the chitosan-propamocarb NPs. The freeze-dried chitosan-propamocarb NPs solution was ground into powder before being bombarded with X-rays for phase analysis. The accompanying XRD pattern was captured using a scanning mode (X 'pert PRO, PAN analytical, Netherlands) with a Cu K radiation tube (= 1.54 A) at 40 Kv and 30 mA. The acquired diffraction pattern was processed by the PDF4 software's standard ICCD library. All procedures were conducted at Nanotechnology and Advanced Materials Central Lab. (NAMCL), Agricultural Research Center (ARC), Egypt.

2.3. Experimental design

2.3.1 Transplanting and treatment

The *in situ* experiments were conducted at the greenhouses section at the Agricultural Experimental and Research Station, Faculty of Agriculture, Cairo University, Cairo, Egypt. Block Design (RCBD) in three replicates was used. The plot was ridged at 2 m long and 1.5 m width with a plot area about 3 m^2. Seedlings were transplanted in winter season 2018/2019. The cucumber was transplanted on 31st October 2018 under plastic greenhouse. The treatments were laid out in a Randomize Complete on two sides of the ridge at 50 cm between them. The number of plants per plot was 10 plants. The examined pesticide was applied at 3 doses: ½ dose, dose, and 2-times dose. The Physical and chemical properties of the soil used in this experiment shown in table (1).

Table 1. Physicochemical characteristics of experimental site soil

Particle Size Distribution (%)	
Coarse sand	6.0
Fine sand	37.0
Silt	22.0
Clay	35.0
Textural class	Clay loam
Chemical properties	
pH (1:2.5)	8.0
EC (dS m^{-1})	0.62
$CaCO_3$ (%)	4.5
Soluble anions (cmolc kg^{-1} soil)	
HCO_3	2.7
SO_4^{-2}	2.5
Cl-	1.0
Soluble cations (cmolc kg^{-1} soil)	
K^+	0.57
Na^+	2.5
Ca^{2+}	1.0
Mg^{2+}	1.7

2.3.2. The vegetative growth measurements

Plant height, a number of leaves per plant, and leaf area (cm^2) were recorded after 21 and 45 days of treatment on five plants from each plot. The leaf area meter was measured by Cl-202 laser area meter (USA).

2.3.3 Yield and its components

Early yield and weight of fruits of 1st and 2nd harvesting were recorded (g/m^2). In addition to total yield, the weight of fruits at all harvesting times (g/m^2) was quantified.

2.3.4 Fruits quality

Average fruit weight, length, and diameter were recorded. Also, Total soluble solid (TSS) has been determined using a digital refract meter (PR 101, CO. Ltd. Tokyo Japan) and firmness (Kg/F) was measured by using Force Gauge model M4-200, USA. All parameters were noted at the 2nd harvesting 10 fruits picked at random from each plot.

2.3.5 Leaves pigments content

Chlorophyll A (*Chl. A*), B (*Chl. B*), and carotenoids (*Carot*.) were assessed after 2, 4 and 6 weeks of treatment date. The Hiscox and Israelstam (1979) procedure was followed. Five ml of dimethyl sulphoxide (DMSO) were added to a test tube containing 10 mg of leaf tissue in fraction. By overnight incubation at 65 ^oC, chlorophyll and carot. were removed into the fluid without grinding. The absorbance was determined using Shanghai Lab-spectrum instrument Co., Ltd Model, Alpha-1102 at wavelengths: 644 and 662 nm for chlorophyll content, and 470 nm for *Carot*. content.

The total chlorophyll: *Chl. A*, *Chl. B* and total *Chl.* were calculated by (Arnon equation 1949), while equation of Cañal *et al*. (1985) was used to calculate *Carot.* as follows:

Arnon equation:

$$Chl.\ A = 12.7 \times A_{662} - 2.69 \times A_{644} \text{ (mg /g fresh weight)}$$

$$Chl.\ B = 22.9 \times A_{644} - 4.68 \times A_{662} \text{ (mg /g fresh weight)}$$

$$Chl.\ A+B = 20.2 \times A_{644} + 8.02 \times A_{662} \text{ (mg /g fresh weight)}$$

Cañal equation:

$$Carot.\ (mg\ L^{-1}) = \frac{A_{470} - 1.28\ (Chl.\ A) + 56.7\ (Chl.\ B)}{256 \times 0.906}$$

2.3.6 Cell Culture

An Egyptian company for the production of vaccines (EGYPVAC, Cairo, Egypt) established the HepG2 cell line. Before usage, the cells were incubated at 37 °C in a 5% CO2 environment in a standard medium composed of DEMEM with 10% (v/v) foetal bovine serum and 1% (v/v) penicillin/streptomycin. Fresh DEMEM 10% in phosphate-buffered saline (PBS) was used to replace the medium. Sub-culturing was used to keep the cells alive after they had reached a suitable confluence.

2.3.7 Toxicity Test

The cells were seeded at a concentration of 1×10^4 cells/ml into 96-well cell culture plates, and they were cultured under standard conditions for 24 hours to obtain exponential growth. The tested pesticides were applied to the cells at doses ranging from 5.0 to 25.0 µg/ml. The medium was removed after 24 hours of incubation and each plate received 5 µg/ml of MTT reagent, which was then allowed to incubate for 3-4 hours. The formazan crystals were dissolved in 100 µl acidified isopropanol and read at 630 nm by using an ELISA microplate reader (Bio-RAD microplate reader, Japan). Three times, each concentration was tested. To compare the defects in the cells following exposure.

On the light microscope, fields of untreated and pesticide-treated cells were examined. . Cell viability was calculated as follows:

Cell viability% =AbsS × 100/AbsC

Where, Abs S and Abs C were absorbances of the cells incubated with samples and without sample, respectively.

2.3.8 Biochemical Quantifications

In cold (50 mM potassium phosphate buffer pH 7.5, 2.0 mM EDTA) homogenization of the pellets was performed. The homogenate was used as a source for LDH, and lipid peroxidation (LPO) as malondialdhyde (MDA) content.

The activity of LDH was determined using the McQueen (1975) method with sodium pyruvate as a substrate. The activity was expressed as $U \cdot L^{-1}$.

Rice-Evans *et al*. (1996) employed thiobarbituric acid reactive substances (TBARS) as an indicator of LPO.

TBARS was determined by spectrophotometric quantification of the MDA content. An aliquot (250 µl) of cell lysate was mixed with 1ml of 15% (w/v) trichloro acetic acid (TCA) in 25 mM from HCl and 2 ml of 0.37% (w/v) thiobarbituric acid (TBA). After boiling for 10 minutes, the mixture was promptly cooled and centrifuged at 5000 rpm for 5 minutes. At 535 nm, the absorbance was measured. MDA content was determined using an extinction coefficient of 156 mM1, and its concentration was expressed as mM•g1 tissue.

2.4 Statistical analyses

All data obtained were statistically analyzed using the (MSTAT-C) computer software tool. The least significant differences (LSD) approach was employed to examine the variations in treatment means at a 5% level of possibility, as specified by (Snedecor and Cochran, 1981).

3. Results

3.1. Characterization of Chitosan-propamocarb nanoparticles

The physicochemical characterization of the produced chitosan-propamocarb-HCl NPs is shown in Figure (1). The NPs are approximately spherical in shape, have a smooth surface, and have a mean size of 29.98 nm (Figure 1A). Figures 1A, 1B, and 1C show DLS profiles of hydrodynamic diameters in the nanoscale range. The NPs had a size of 24.98 nm and a zeta potential of 45.7 mV. X-Ray diffraction patterns are shown in Figure 1D. Chitosan-propamocarb-HCL NPs exhibited a broad characteristic hump peak beginning from $2\theta = 14°$ to $2\theta = 44°$. The main peak was observed at $2\theta = 22°$.

3.2. Morphological parameters after 30 and 60 days of treatments

Data in Table (2) indicated that, all treatments affected the number of leaves per plant, plant height (cm), and leaf area meter (cm^2) of cucumber plants after 30 days of treatment. No significant variations in No. of leaves/plant between treatments were found, they ranged from 6.417 to 7.25, when compared with the control group (7.667). While, the dose of traditional pesticide exhibited a decrease in plant length (40.13 cm) compared with the control (51.83 cm). No significant differences between ½ dose and 2-Times dose. On the other hand, the dose of nano-form pesticide exhibited a significant decrease (27.83 cm), followed by a 2-times dose (29.63 cm), and ½ dose (38.25 cm). All treatments exhibited significant increases in leaf area in the range 71.70–111.90 cm^2, compared with the control group (56.68 cm^2). The obtained results demonstrated that, the number of leaves was negatively affected by treatment with propamocarb-HCl after 30 days of treatment, while propamocarb-HCl had significant effects on plant height and leaf area.

After 60 days of treatment, no significant differences were obtained in No. of leaves/plant in all treatments (range; 14.33-16.08), compared with control (16.25). All treatments significantly decreased plant length in the range from 90.75 to 101.80 cm, compared with control (117.10 cm). However, all treatments significantly increased leaf area in the range from 125.2 to 157.9 cm^2, compared with control (106.3 cm^2) (Table 3).

3.3. Yields

All treatments of traditional pesticides exhibited no-significant differences in No of fruits/plant (range 2.82-3.713), compared with control (3.807). While, significant increases were noticed for nano-form as follows: 5.673, 4.213, and 3.877 for dose, ½ dose, and 2-Times of dose, respectively. Significant decreases in fruit weight/plant were noticed for treatments of traditional pesticide-inducing mean values: 45.65, 57.40, and 46.62 g for dose, ½ dose, and 2-Times dose, respectively, compared with control (67.35 g). On the other hand, dose of nano-form pesticide exhibited a significant increase (74.48 g), but other

doses: ½ and 2-Times significantly decreased fruit weight (mean; 59.88 and 51.81 g). Finally, all treatments exhibited significant decreases in total yield in the range (190.20-832.60 Kg/m^2), compared with control group (805.70 Kg/m^2) (Table 4).

3.4. Effects on fruits quality

All treatments of traditional pesticide exhibited a significant decrease in fruit length with mean values: 11.03, 12.60, and 11.57 cm for dose, ½ dose, and 2-Times dose, respectively, compared with control (14.72 g). However, ½ dose and 2-Times dose of nano-form pesticide exhibited significant increase 16.47 and 15.24 cm. On the other hand, all treatments of traditional and nano-form of the examined pesticide exhibited a decline in fruit diameter ranging from 12.94 to 28.11 mm, compared with control (29.78 mm). Slight increases were induced in fruit firmness of all treatments between 2.183 and 2.977, compared with 2.153. Also, non-significant differences were obtained between treatments in TSS values in the range 2.890- 3.056 brix, compared with control 2.677 brix (Table 5).

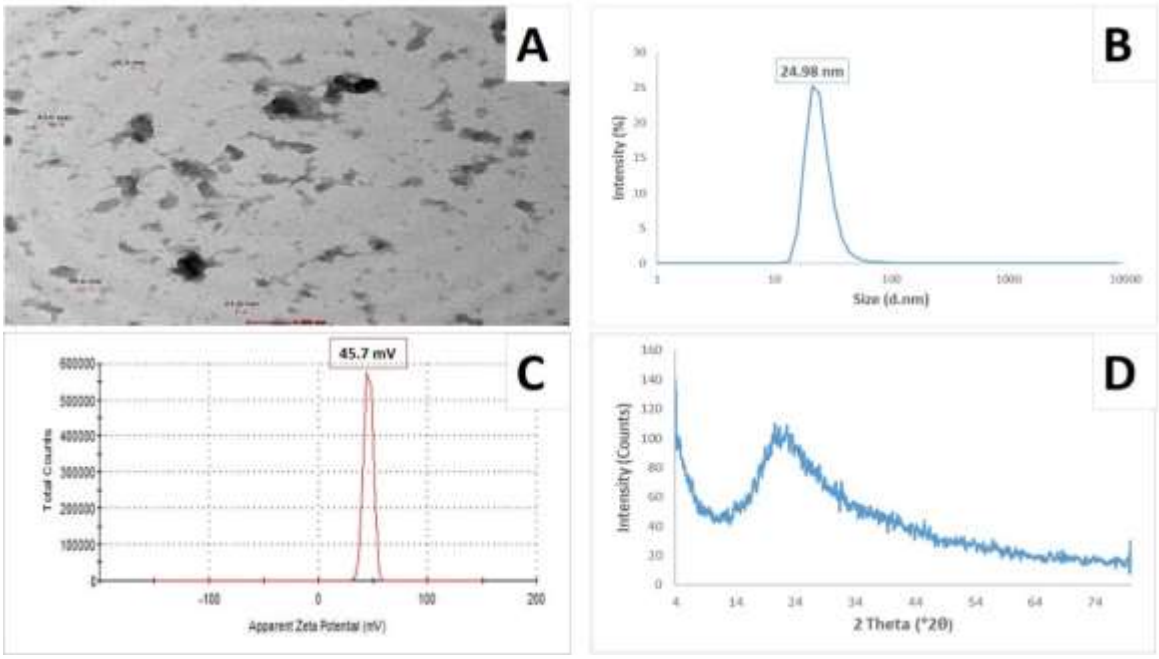

Fig. 1. Characterization of chitosan-propamocarb NPs. (A): HR-TEM image displaying an approximately spherical shape with an average size of 29.98 nm, (B): produced a nano-form particle size distribution with an average size of 24.98 nm, (C): Zeta potential revealing the surface charge, 45.7 mV and (D): XRD pattern analysis showing the creation of chitosan-propamocarb NPs at certain diffraction objects

Table 2. Effect of propamocarb-HCl and its nano-derived form on morphological parameters of the cucumber plants after 30 days of treatment.

Pesticide	Dose (cm/100 L H_2O)	No. of leaves per plant	Plant length (cm)	Leaf area meter (cm^2)
Traditional	**Dose**	7.250ab	40.13b	79.03de
	½ Dose	6.917ab	31.88bcd	95.11bc
	2- Times dose	6.750ab	31.50bcd	108.2ab
Nano-form	**Dose**	6.417b	27.83d	108.5ab
	½ Dose	6.417b	38.25bc	111.9a
	2- Times dose	6.750ab	29.63cd	71.71ef
	Control	7.667a	51.83a	56.68f
LSD $_{0.05}$		1.018	9.878	15.84

- All values are means of three replicates (mean ± SD).
-Means in every column followed by the same letter are not significant at P > 0.05.
*Dose: Recommendation stated by Egyptian Ministry of Agriculture.

Table 3: Effect of propamocarb-HCl and its nano-derived form on morphological parameters of the cucumber plants after 60 days of treatment.

Pesticide form	Dose (cm/100 L H_2O)	No. of leaves per plant	Plant length (cm)	Leaf area meter (cm^2)
Traditional	**Dose**	15.08a	90.75ab	136.7bc
	½ Dose	16.08a	101.8ab	136.0bc
	2- Times dose	15.83a	92.42ab	127.2c
Nano-form	**Dose**	14.33a	94.17ab	145.9ab
	½ Dose	15.42a	93.08ab	157.9a
	2- Times dose	15.67a	97.75ab	125.2c
	Control	16.25a	117.1a	106.3d
LSD 0.05		NS	28.98	13.81

- All values are means of three replicates (mean ± SD).
-Means in every column followed by the same letter are not significant at P > 0.05.
*Dose: Recommendation stated by Egyptian Ministry of Agriculture.

Table 4: Effect of propamocarb-HCl and its nano-derived form on the yield of fruits.

Pesticide form	Dose (Cm/100 L H_2O)	No. of fruits per plant	Average fruit weight (g)	Total yield (kg m^{-2})
Traditional	**Dose**	3.713b	45.65c	428.6cd
	½ Dose	2.820b	57.40bc	439.8cd
	2- Times dose	3.097b	46.62c	190.2e
Nano-form	**Dose**	5.673a	74.48a	832.6a
	½ Dose	4.213ab	59.88abc	760.2ab
	2- Times dose	3.877b	51.81bc	358.7de
	Control	3.807b	67.35ab	805.7ab
LSD $_{0.05}$		1.504	16.42	220.3

- All values are means of three replicates (mean ± SD).
-Means in every column followed by the same letter are not significant at P > 0.05.
*Dose: Recommendation stated by Egyptian Ministry of Agriculture.

Table 5: Effect of propamocarb-HCl and its nano-derived form on fruits quality.

Pesticide form	Dose (Cm/100 L H_2O)	Fruit length (cm)	Fruit diameter (mm)	Fruit firmness (N)	TSS (Brix)
Traditional	**Dose**	11.03e	23.56bc	2.423abc	2.967a
	½ Dose	12.60de	21.94c	2.183bc	3.057a
	2- Times dose	11.57e	22.39bc	2.440abc	2.900a
Nono-form	**Dose**	14.24bcd	24.67abc	2.410abc	2.890a
	½ Dose	16.47a	28.11ab	2.977a	2.967a
	2- Times dose	15.24ab	27.55abc	2.747ab	3.047a
	Control	14.72abc	29.78a	2.153c	2.677a
LSD $_{0.05}$		2.074	5.746	0.5782	NS

- All values are means of three replicates (mean ± SD).
-Means in every column followed by the same letter are not significant at P > 0.05.
*Dose: Recommendation stated by Egyptian Ministry of Agriculture.

3.5. Leave pigments

After 15 days of treatment the data indicated that significant declines in *Chl. A* was obtained in all doses of traditional pesticide in the range 11.0622-13.0963 mg /g fresh weight, compared with control (15.699 mg /g fresh weight). Similar declines were obtained in all doses of nano-form, where the range was 12.5058-14.338 mg /g fresh weight. This finding was stated in *Chl. B* arising the ranges 3.022-4.188 mg /g fresh weight and 3.35-4.473 mg /g fresh

weight for traditional pesticide, and its nano-form. The total *Chl.* showed the above pattern arising the range 14.084 – 18.811 mg /g fresh weight, compared with control (22.088 mg /g fresh weight). Similarly, *Carot*. showed significant declines in all treatments (0.682-1.0203 mg /g fresh weight), compared with control (1.4821 mg /L) (Table 6).

Table 6: Effect of propamocarb-HCl and its nano-derived form on leaf pigments after 15 days of treatment.

Pesticide form	Dose (Cm/100 L H_2O)	Ch. a (mg/g)	Chl. b (mg/g)	Total Chl. (mg/g)	Carotenoids (mg/g)
Traditional	**Dose**	12.1952 e f	3.7547 b c d	15.950 d	0.8555 b c d
	½ Dose	13.0963 c d e	4.1883 b c	18.080 b c	0..9531 b c
	2- Times dose	11.0622 f	3.0220 d	14.084e	0.6820d
Nano-form	**Dose**	14.3384 b	4.4729b	18.811 b	1.0203 b
	½ Dose	12.5058 d e	3.3517 d	15.858d e	0.7549 d
	2- Times dose	13.8919 b c	3.7038 c d	16.800c d	0.8389c d
	Control	15.6989 a	6.3888 a	22.088 a	1.4821a
LSD $_{0.05}$		0.8291	0.5065	1.2632	0.1206

- All values are means of three replicates (mean ± SD).
-Means in every column followed by the same letter are not significant at P > 0.05.
*Dose: Recommendation stated by Egyptian Ministry of Agriculture.

After 30 of treatment the ½ dose of traditional pesticide exhibited a slight decrease in *Chl. A* (7.000 mg/g), compared with control 7.313 mg /g fresh weight, followed by dose (6.223 mg /g fresh weight). On the other hand, nano-form pesticide exhibited significant increases as follows: 10.396, 11.200, and 10.435 mg /g fresh weight for dose, ½ dose, and 2-Times dose, respectively, compared with control. The same pattern was obtained in the case of *Chl. B*; where traditional pesticide exhibited significant declines 1.468, 1.797, and 1.597 mg /g fresh weight for dose, ½ dose, and 2- Times dose, respectively, compared with control (1.949 mg /g fresh weight). However, significant increases were noticed for the above doses arising the mean values: 2.731, 3.037, and 2.628 mg /L. Similar finding was obtained for total *Chl.*, where traditional pesticide ranged from 6.729 to 8.797 mg /g fresh weight, compared with control (9.263 mg /g fresh weight). Nano-form pesticide exhibited significant increases 13.127, 14.237, and 13.066 mg /g fresh weight for the above doses, respectively. The same pattern was recorded for *Carot.* level (Table 7).

Table 7: Effect of propamocarb-HCl and its nano-derived form on leaf pigments after 30 days of treatment.

Pesticide form	Dose (Cm/100 L H_2O)	Ch. a (mg/g)	Chl. b (mg/g)	Total Chl. (mg/g)	Carotenoids (mg/g)
Traditional	**Dose**	6.223d	1.468c	7.691d	0.327c
	½ Dose	7.000c	1.797bc	8.797c	0.403bc
	2- Times dose	5.162e	1.567bc	6.729d	0.356bc
Nano-form	**Dose**	10.396b	2.731a	13.127b	0.614a
	½ Dose	11.200a	3.037a	14.237a	0.685a
	2- Times dose	10.438b	2.628a	13.066b	0.588a
	Control	7.313c	1.949b	9.263c	0.439b
LSD 0.05		0.4975	0.291	0.789	0.069

- All values are means of three replicates (mean ± SD).
-Means in every column followed by the same letter are not significant at P > 0.05.
*Dose: Recommendation stated by Egyptian Ministry of Agriculture.

Table 8: Effect of propamocarb-HCl and its nano-form on leaf pigments after 45 days of treatment.

Pesticide form	Dose (C/100 L H_2O)	Ch. a (mg/g)	Chl. b (mg/g)	Total Chl. (mg/g)	Carotenoids (mg/g)
Traditional	**Dose**	7.883a	1.833a	9.716a	0.407a
	½ Dose	2.901f	0.749f	3.650f	0.168f
	2- Times dose	5.148c	1.187c	6.335c	0.264c
Nano-form	**Dose**	4.497d	1.180c	5.677d	0.265c
	½ Dose	4.247d	1.053d	5.300d	0.236d
	2- Times dose	3.451e	0.875e	4.326e	0.196e
	Control	2.944f	0.748f	3.692f	0.169f
LSD 0.05		0.333	0.058	0.374	0.013

After 45 of treatment the all treatments exhibited a significant increase in *Chl. A*, except ½ dose of traditional pesticide which exhibited a decrease in *Chl. A* level (2.901 mg /g fresh weight), compared with control (2.944 mg /g fresh weight). The all treatments exhibited significant increase in *Chl. B* levels in the range (0.749-1.833 mg /g fresh weight), compared with control (0.748 mg /L). The same pattern was noticed in the case of total *Chl.* and control (Table 8). The previous results are very similar to the results in the previous table, which means that the treatments negatively affected the characteristics of the *Chl. A*, *Chl. B*, total *Chl*., and Carot. (mg/g fresh weight). when treated after 45 days of treatment cucumber plants, which means that treatment of propamocarb-HCl reduced *Chl. A*, *Chl. B*, total *Chl*., and *Carot*. (mg/g fresh weight) of cucumber plants. The results also are in accordance with the opinions of many investigators which found that foliar application of propamocarb-HCl decreased the *Chl. A*, *Chl. B*, Total *Chl.*, and *Carot.* (mg/g fresh weight) of cucumber plants.

3.5. Toxicity responses

The tested pesticide, propamocarb-HCl, and its nano-derived form showed significant decrease in generation of the cells. Their values of EC_{50} were 6.35 and 7.74 µg/ml for traditional pesticide and its nano-derived form with 1.22-folds.

3.5.1. LDH

Lactate dehydrogenase (LDH) was used as useful biomarker to assess cell damage. In the current research, propamocarb-HCl showed a significant increase in the enzyme activity (186.19 U. L^{-1}), compared with control (64.77 U. L^{-1}). While its nano-derived form showed non-significant increase in the activity (64.76 U. L^{-1}) (Figure 2).

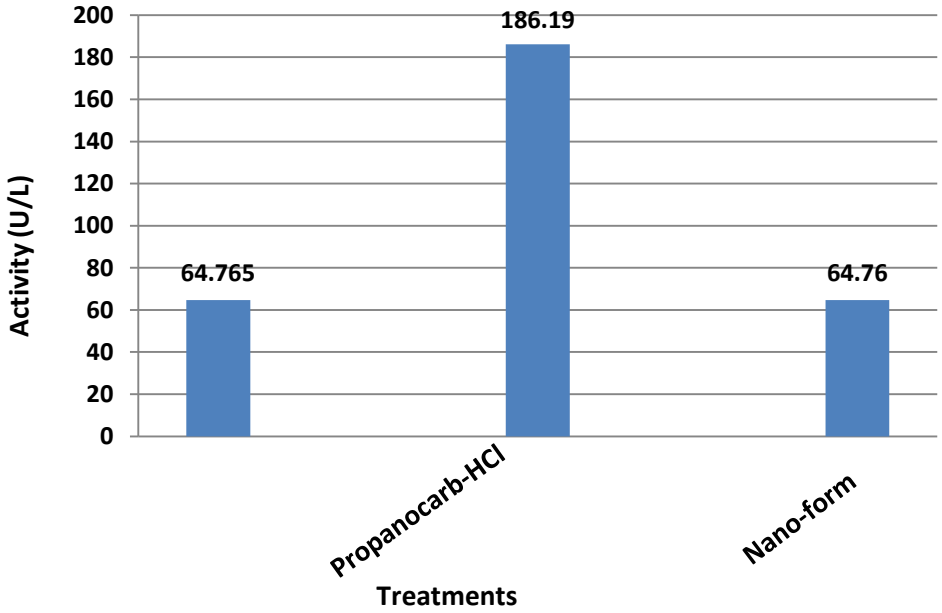

Fig. 2. Activity of lactate dehydrogenase (LDH) (U. L^{-1}) in HepG2 cell line exposed to Propamocarb-HCl and its nano-derived form for 48 hr.

3.5.2. MDA

As described above, propamocarb-HCl induced a significant increase in MDA level (1.205 mM. g^{-1} tissue), compared with control cells (0.426 mM. g^{-1} tissue). However, its nano-derived form did not induce increase in MDA levels (0.433 mM. g^{-1} tissue) (Figure 3).

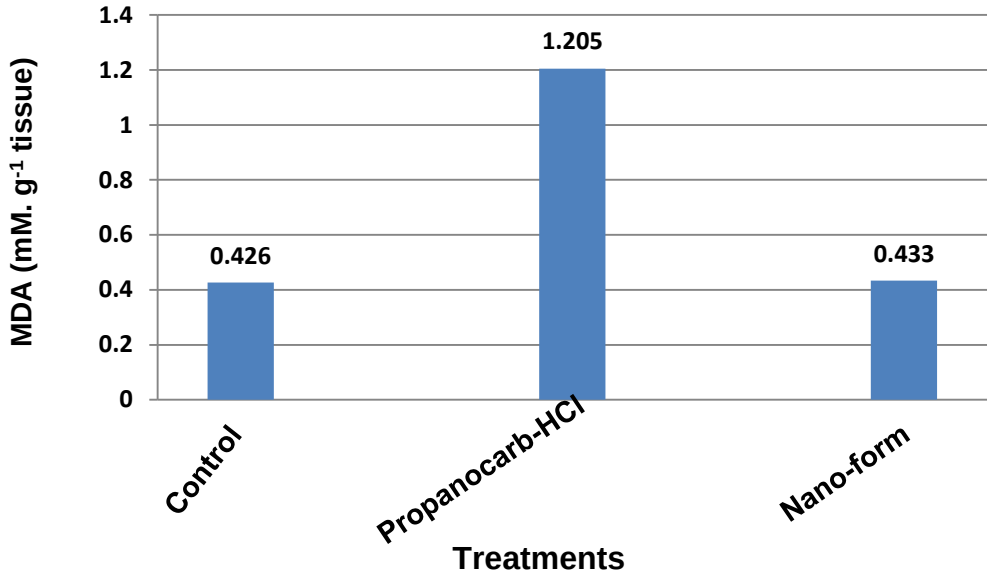

Fig. 3. Levels of malondialdhyde (MDA) (mM. g^{-1} tissue) in in HPG2 cell line exposed to Propamocarb-hydrochloride and its nano-derived form for 48 hr.

4. Discussion

We show how a pesticide used extensively in Egypt's agriculture has cytotoxic effects. Moreover, it was examined pesticide and its nano form under low environmental and physiological concentrations that can be detected in groundwater systems near agricultural crops. The influence of the investigated pesticide and its nano-forms obtained in this work do not appear to be closely tied to the mode of action in controlling insects, fungi or weeds. Also, we have highlighted the effect of the pesticide propamocarb-HCl, both in traditional and nano form, on cucumber plants under greenhouse conditions, as well as the effects of the pesticide in vitro on a human cell line.

Understanding plant growth is a very important step to analysis plant performance and productivity that shows the different strategies a plant employs to survive in conditions that are limited by several factors in the experiment, The yield is ultimately impacted by physiological processes and various enzymatic and non-enzymatic antioxidants as a result of pesticide residues in the plant, fruits, vegetables, and various non-target organisms (Stevens *et al.*, 2008).

Treatment with propamocarb-HCl reduced the vegetative growth of the cucumber plants. In order to protect plants from pests, pesticides are used across the world. However, their use also results in plant toxicity, which negatively impacts plant growth and development. Pesticides can control or destroy plants via a variety of mechanisms, involve biological process inhibition such as cell division, photosynthesis, mitosis, root growth, enzyme function, or leaf formation; interference with the synthesis of pigments, proteins, or DNA; destruction of cell membranes; or promoting hazards growth. Application of pesticides ahead of time in germination may be effect on plant growth and lead to changes in the biochemical components (William *et al*., 1995).

The obtained results are also in accordance with the opinions of many investigators who discovered that propamocarb-HCl foliar application decreased the vegetative growth of the cucumber plants, pesticide stress causes significant plant damage, resulting in poor agricultural output. Seed germination is a highly complex and delicate process that involves numerous physiological activities at the metabolic level. (Singh and Sahota, 2018).

On the other hand, the present results regarding the total yield and the quality characteristics of the fruits of cucumber plants "fruit length, fruit diameter, fruit firmness, and TSS" indicated that the use of propamocarb-HCl by the nano form had the slightly positive effect compared to the other treatments and the control treatment, which means that the use of it has a significant positive effect and that the nano form provides better and greater productivity for the cucumber crop. In the same trend, many reports suggested that foliar sparing of propamocarb-HCl can

stimulate the yield and improve the quality of cucumber fruits. Pesticide accumulation by the plant inhibited plant growth and caused metabolic problems. (Sharbels *et al*., 1997).

When we studied photosynthetic pigments found significant declines in *Chl. A* in all doses of traditional and nano form pesticide after 15 days of treatment and this result is agreement with Hall and Sivakumaran, (1978) noticed that when pesticides are applied too early, they may impair plant germination, altering biochemical and physiological processes as well as different enzymatic and non-enzymatic antioxidants, which ultimately affects plant yield and residues in non-target organisms. Also, (Tort and Turkyilmaz, 2003) reported that the decline of photosynthetic pigment particles may reduce the nutritional value of the pepper plant. In light of the fact that vitamin production occurs in chloroplasts. Our results are also in agreement with (Khanday, 2022) found that all pesticides are proven to be toxic to the accumulation of Chlorophyll in the wheat leaves. Both *Chl. A* and *Chl. B* decreased in direct proportion to the concentration of the applied pesticides. Previous research has revealed that any stress-related stimulus causes chloroplast malfunction and photosynthetic damage (Xing *et al.*, 2013). While, Wang *et al*. (2018) suggest that the reduction of photosynthesis could be the reason for the decline in photosynthetic pigments. For example, metribuzin inhibit photosynthesis by blocking electron transfer from the Q complex to the blastocinone in photosystem II, thus preventing the reduction of NADPC required for CO fixation. Also, Abdel-Gawad (2001) reported that pesticides affected the growth of crops, causing a decrease in their yield and quality due to their phytotoxicity. The use of pesticides above permissible amounts may lead to oxidative stress and cause oxidative damage in non-target host plants (Shakir *et al.*, 2018).

The responses of the examined human cells in our study to the possibility harmful effect of propamocarb-HCl and its nano-form differed significantly. The cell line is frequently used as a cytotoxic model to investigate pesticides at concentrations that may be received to human and other mammalian cellular systems. In fact, the utilisation of cell lines in conjunction with biomonitoring data could allow for a comprehensive understanding of environmental metal/chemical toxicity (Nakadai *et al*., 2006). Propancarb-HCl and its nano-form have cytotoxic effects on human cells and may be effective in vivo over long-term exposure at low dosages for humans, according to the study, Pesticides inside the cell cause intracellular reactive oxygen species (ROS) and cell disorganization (Matés *et al*., 2010). Toxic compounds raise the oxygen level within the cell, inhibiting numerous cell organelles and promoting apoptosis. (Valencia and Cochevar, 2006). It is also known that oxidative stress can disrupt cellular membranes by changing tight junction molecules. (Lee *et al*., 2004; Hashimoto *et al*., 2008). Pesticides such lambdacyhalothrin, teflubenzuron, fenitrothion, fipronil, deltamethrin, and fenitrothion caused functional changes in the epithelial Caco-2 cell layer in connection to their pro-oxidative activities (Ilboudo *et al.*, 2014).

5. Conclusion

The fungicide propamocarb-HCl and its nano-form were examined for their phytotoxic effects on cucumber plant growth, yield quantity, and quality, as well as most physiological and biochemical aspects. The increase in the application has been shown to be related to the severity of the phytotoxic effect of the treated plant. On the other hand, the traditional form was greater phytotoxic than the nano-form. Also, this pattern was similar with that obtained cytoxicity on HepG2 cell line. Such findings may provide a lot of biosafety profiles of nano-formulation practices in the agricultural sector. So, more toxicological studies must be done, before the decision this use of nano-substances in the environment.

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