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Research article

Insecticide potentiality of rice case (chaff) particle

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ABSTRACT

Chaff (the outermost protective layer of rice grain) in nano and ground sized particles was bio-assessed to explore its insecticidal effects against the greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae). These particles induced remarkable disturbances in the biological patterns of the greater wax moth. These were cleared as they exhibited insecticidal impact on both juvenile and adult stages, their impacts on larval, pupal and adult death, the formation of morphogenetic features (permanent larvae and larval-pupal intermediates), their negative influence on the adult emergence and the potentialities of the applied concentrations to hinder the larval weight gain. The recorded LC_{50} values proved more larvicidal potency of the nano-sized particle than that of the ground rice chaff one. The transverse sections in the midgut of the 20 days old larvae (both treated and untreated ones) confirmed the histopathological deformations of the examined particles. The high siliceous content of the chaff particle may be responsible for such recorded disturbances.

1. Introduction

Agriculture expansion is an urgent demand in order to bridge the global food gap induced due to the overestimated population growth. Insect pests and their induced losses are considered as one of the main detrimental factors that face agriculture process. Accordingly, the strategies, tactics and efforts that intended for insect pest control mount great concern in order to alleviate their dangerous impacts. At the beginning of the 20th century classical chemical insecticides had the lion's share in this regard. Although pesticide application fulfilled satisfied results in decreasing the losses of most of the economic pests, their negative impacts on both human and environment (soil, marine, wild life…*etc.*) following the long run practices besides the appearance of resistant strains (Mosallanejad and Smagghe, 2009; Sharifian et al., 2012; Sparks et al., 2012) were the main motivator to find out less risky alternatives or to reformulate these conventional chemicals to achieve their missions in safer ways (Savary et al., 2012). The dawn of 21st century saw a global revolution in the field of Nano-technology science that played and still playing vital roles in human welfare fulfillment (Scott and Chen, 2002; Salamanca-Buentello et al., 2005; Torchilin, 2006; Chena and Yada, 2011; Manjunatha et al., 2016). In agriculture, among all contributions of nano-sized particles in the enhancement of agricultural productivity (Corradini et al., 2010; Anders and Glotzer, 2012), nano-pesticides not only adopted more efficacies than their normal sized products but also revealed a matching state with the commitments of the Environmental Protection Agency (EPA) in terms of declining the environmental pollution due to conventional pesticides besides their high potentiality against pests (Choudhary et al., 2010 and Gopal et al., 2012). In this concern, a lot of studies had been addressed different forms of the nano-sized particles in order to be ready to engage in the pest management approach. Nano-encapsulating agents were used in order to ensure the arrival of the herbicides to the desired target (Chinnamuthu and Kokiladevi, 2007). This nano protective coats were also used to encapsulate insecti-, fungi- and/or nematicides for the controlled release and consequently less environmental and soil pollution (OECD and Allianz, 2008). The linkage between paraquat (widely used herbicide) and alginate/chitosan nano-particles according to Silva et al. (2011) minimized the negative influence of the herbicide through a controlled releasing technique. Another environmentally important issue was encountered by Susha et al. (2009) through their obtained results that adopted the detoxification of the herbicidal residues from the soil using the nano-technology.

Rice husk or Chaff is the outermost cover of the rice grain that acts as a protective shelter for the embryo. Actually, it is an un-edible part and considers as a sort of grain waste. Chaff is a multi-purpose material, which is recycled to be exploited as a soil fertilizer or mixed with animal diet as a supplement or used as a planting medium (Ummah et al., 2015). The potentiality of rice chaff as an absorber in the desalination process of sea water to get fresh water (Ummah et al., 2014; Syarif et al.,

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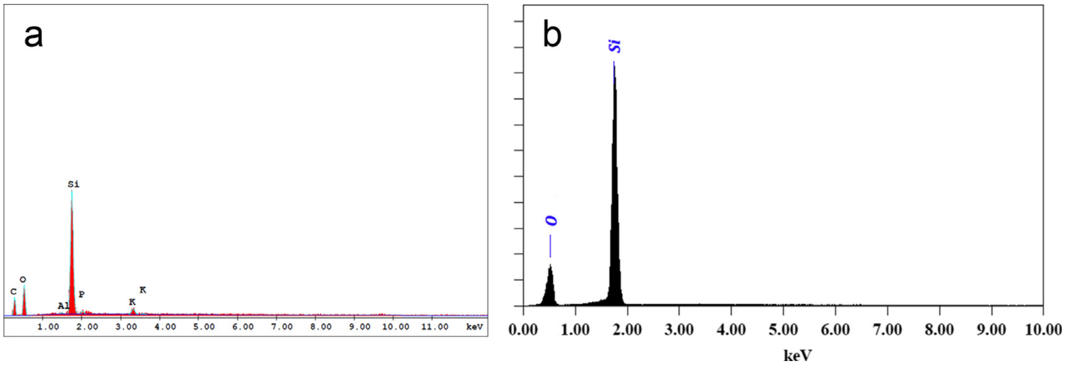

Figure 1. Energy-dispersive X-ray spectrum for (a) GC and (b)NS.

2015) and its role in the detoxification of aqueous solutions through its capability to adsorb either cadmium and zinc (Srivastava et al., 2008) or pesticide mixture (Saha et al., 2014) are two vital missions that rice chaff succeeded to act. The current study had been designed to examine the insecticidal potentiality of rice chaff particles that prepared in two forms [ground rice chaff (GC) and nano-sized (NS) silica] using the greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae), as a lab. model.

2. Material and methods

2.1. Laboratory production of greater wax moth, Galleria mellonella

Egg cards of the greater wax moth, *G. mellonella*, had been obtained from the rearing insectaries of the Entomology unit, Plant Protection Department, Desert Research Center. Collected egg cards were put in plastic jars (1 kg size) loaded by about 100 g of the larval nutritional diet previously described by Bhatnagar and Bareth (2004). Hatched neonate larvae had been fed on the diet till reached the full grown form. These full grown caterpillars pupate within corrugated cardboard that previously put in the larval rearing jars. Emerged moths were transferred to another glass chimney for egg deposition. The upper chimney's opening was covered by double layered tissue paper and its edge by a piece of paper under which mated female moths put its eggs. The upper and lower openings of the chimney had been covered by muslin and secured by rubber band. *G. mellonella* rearing program was incubated under 30 $\pm$ 1 $^{\circ}$C and 80 $\pm$ 5 % RH. conditions.

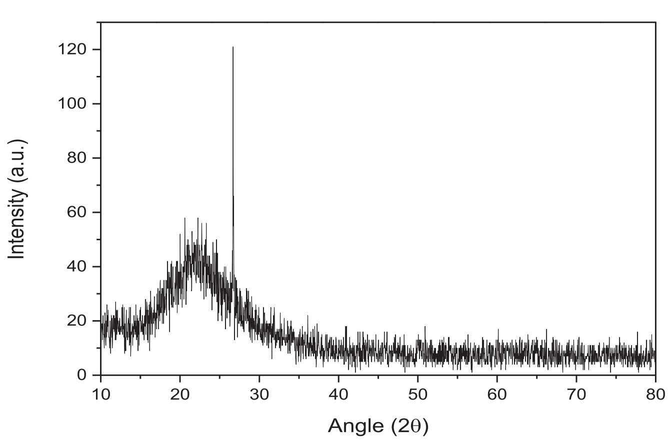

Figure 2. X-ray diffraction spectrum for NS.

2.2. Treatment of rice chaff samples

Rice chaff was supplied from rice mills, Sharkia Governorate, Egypt. Lab-grade 34% hydrochloric acid was supplied by El Nasr Co., Cairo, Egypt.

The rice chaff was dried at 120 $^{\circ}$C for 2 h to eliminate any humidity, then well shredded using a 150-watt stainless steel blade grinder to obtain ground rice chaff (GC). For the preparation of nano-sized silica (NS); part of the ground rice chaff was refluxed with 17% hydrochloric acid solution as a leaching agent (1:10 w/v) for 1 h. The acidified rice chaff was collected, washed with distilled water repeatedly and left to dry at 100 $^{\circ}$C overnight. The dried product was subsequently annealed in a muffle furnace at 650 $^{\circ}$C and kept for 2 h in ambient air, and eventually subjected to rapid cooling.

2.3. Characterization of treated rice chaff

Phase composition of the nano-sized silica (NS) was identified by X-ray diffraction technique using the PHILIPS® MPD X'PERT diffractometer. Fully automated BELSORP-mini II adsorption desorption analyser was employed in order to study the surface characteristics of the obtained samples. The surface morphology and semi quantitative element analysis of the two treated rice chaff samples were observed using FEI Quanta 250 FEG scanning electron microscope/energy dispersive X-ray spectroscopy (SEM/EDX).

Additionally, a JEOL JEM-1230 transmission electron microscope (TEM) was utilized to investigate the form and quantitatively measure the obtained particle size.

2.4. Bioassay examination

To evaluate the insecticidal efficacy of the rice chaff particles, 3 concentrations of each form (GC and NS) (150, 200 and 300 ppm) were prepared. One hundred and fifty grams of the artificial diet were weighted and well homogenized with 2.5 g of each prepared concentration, *i.e.* 0.05 g/3 g diet. Such amount of diet had been equally distributed on 50 glass tubes, *i.e.* 3 g. diet/each tube and 50 replicates/each concentration. One newly hatched larva was transferred to each tube using fine hairbrush then plugged by cotton stopper. Similar amount of untreated diet was weight and distributed on 50 glass tubes with one neonate larva per each tube to serve as check treatment. Both treated and check experiments underwent 48 h investigation schedule to determine the death and longevity of larval, pupal and adult stages. The observed percentages of death (both larval and overall) were corrected according to Abbott's formula (Abbott, 1925):

$$\text{The corrected percent} = \frac{\text{Observed} - \text{Control}}{100 - \text{Control}} \times 100$$

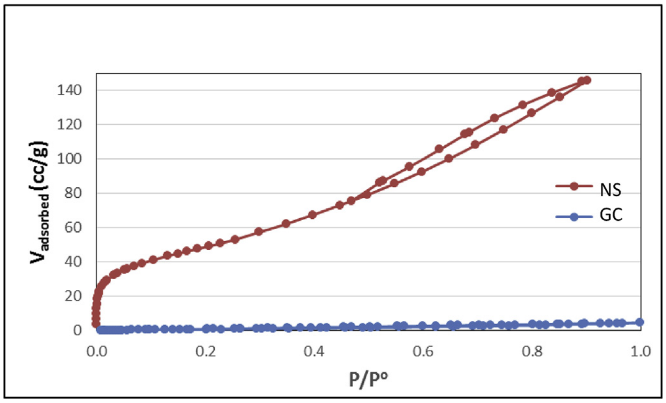

Figure 3. N_2 Adsorption – desorption isotherms for the two treated rice chaff samples.

Table 1. Textural parameters from N_2 adsorption-desorption analysis of the treated rice chaff samples.

Sample	BET surface area (m^2/g)	Average pore diameter (nm)	Total pore volume (cc/g)
GC	3.46	14.12	0.012
NS	179.7	5.03	0.323

The LC_{50} values (the lethal concentration that kill 50% of the larval population) for both particles had been calculated according to Finney (1971).

The influence of rice chaff particles on the larval weight gain was assessed through weighted the 20 days old larvae in treated and check trials and the obtained date was subjected to one way ANOVA analysis using SPSS PASW Statistics ver. 18.

2.5. Histological technique

This experiment addressed the histological deformations of the larval midgut following the treatment. Ten newly hatched larvae were individually treated as previously described with the calculated LC_{50} values of GC and NS particles and another cohort was used as check treatment. Transverse sections (T.S.) in the midgets of the 20 days old larvae had been carried out according to Disbrey and Rack (1970). Light microscope was used to examine the obtained sections.

3. Results

Figure 1 represents EDX spectrum for both GC and NS samples. The XRD pattern of nano-sized silica (NS) is exhibited in Figure 2.

Figure 3 illustrates the N_2 adsorption-desorption isotherm of the two prepared samples (GC and NS). Other surface texture parameters are summarized in Table 1.

The morphology, particle's form and dimensions of the prepared samples were confirmed using SEM (Figure 4 a and b) and TEM (Figure 4c), respectively.

Treated particles of rice chaff induced remarkable disturbances in the biological patterns of the greater wax moth. Tables 2 and 3 illustrated the effects on both juvenile and adult stages. In this regard, periodical larval death was noticed till the 28th day of the treatment commencement, which coincided with the completion of larval stage in the check trial. Treated larvae that didn't undergo pupal transformation and died as worms were documented as permanent larvae. As the total larval death recorded 10 individuals at the highest applied concentration (300 ppm) of the nano-sized particle (NS), such value was duplicated at the lowest applied ones. The situation showed inverse pattern upon considering the applied concentrations of the ground rice chaff sample (GC). Where, the highest applied concentration met the maximum total larval death. Another worthy-note point was the minor larval death 2 days post treatments with fluctuated death patterns in a time dependent manner at the all applied concentrations (Tables 2 and 3). As shown in Figure 5, LC_{50} values recorded ~118.6 and 352.1 for both NS and GC particles, respectively.

The subsequent addressed parameter was the total pupal death, which had been got from the algebraic summation of the dead pupa (P) + the larval-pupal intermediate (LP) (Figure 6). Tables 2 and 3 revealed different impacts of the two applied particles on the total pupal death. As the total pupal death exhibited inverse relationship with the applied concentrations of the NS (the assayed 150 ppm concentration fulfilled higher pupal death than the others), the GC samples behaved

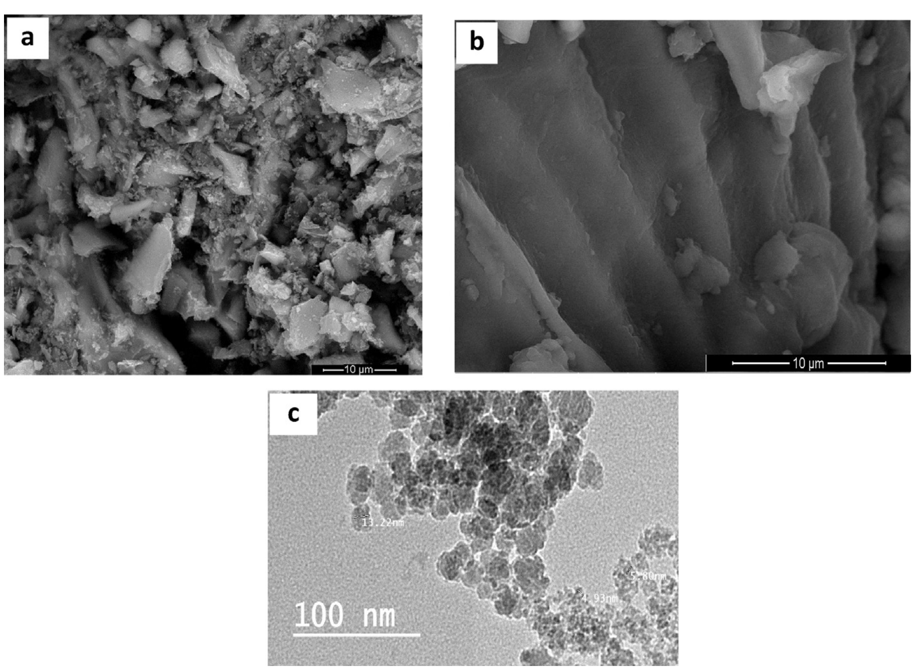

Figure 4. (a and b): Scanning electron microscope images NS and GC, respectively and (c) Transmission electron microscope image for NS.

Table 2. Potentialities of the nano-sized rice chaff particle (NS) on the juvenile and adult death, the malformed individuals and the emerged adults of the greater death moth, *Galleria mellonella*.

	Number of dead larval after (days)						Total larval death			P.	LP.	Total pupal death	Viable pupation	Emerged adults			Overall death		
	2^{nd}	7^{th}	14^{th}	21^{st}	28^{th}	Perm.	Obs.	% obs.	% corr.					Normal	Deformed	Total adult death	Obs.	% obs.	% corr.
C.	1	1	1	0	0	0	3	6	0	1	0	1	46	46	0	0	4	8	0
150 ppm	0	10	5	0	0	5	20	40	36.17	10	7	17	13	10	3	0	37	74	71.74
200 ppm	2	5	5	3	0	5	20	40	36.17	10	0	10	20	13	2	5	35	70	67.39
300 ppm	0	7	0	0	3	0	10	20	14.89	5	7	12	28	18	5	5	27	54	50

Table 3. Potentialities of the ground rice chaff particle (GC) on the juvenile and adult death, the malformed individuals and the emerged adults of the greater death moth, *Galleria mellonella*.

	Number of dead larval after (days)						Total larval death			P.	LP.	Total pupal death	Viable pupation	Emerged adults			Overall death		
	2^{nd}	7^{th}	14^{th}	21^{st}	28^{th}	Per.	Obs.	% obs.	% corr.					Normal	Deformed	Total adult death	Obs.	% obs.	% corr.
C.	1	1	1	0	0	0	3	6	0	1	0	1	46	46	0	0	4	8	0
150 ppm	0	8	5	0	2	5	20	40	36.17	5	3	8	22	17	5	0	28	56	52.17
200 ppm	0	3	2	5	5	0	15	30	25.53	10	3	13	22	10	12	0	28	56	52.17
300 ppm	3	10	4	0	3	7	27	54	51.06	8	5	13	10	5	3	2	42	84	82.61

Per.: Permanent larvae, didn't undergo pupal transformation and died as worms, P: dead (deformed) pupae, LP.: Larval pupal intermediate (malformed feature), Total adult death: Died moths 48 h post emergence, Overall death = Total larval death + Total pupal death + Total adult death.

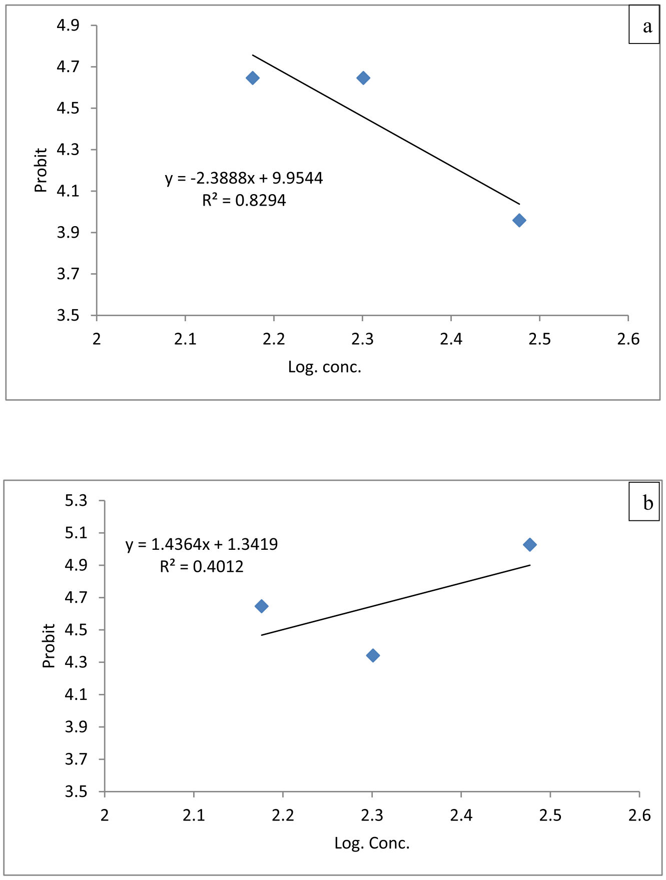

Figure 5. Concentration/mortality regression lines for *Galleria mellonella* treated with rice chaff particles a: NS and b: GC.

proportional trend. The impacts of the rice chaff particles on both larval and pupal death besides the appearance of malformed features played an obvious role in adjusting the formation of viable pupae from which moths emerged. The viable pupae recorded 46 ones at the check treatment whereas the maximum ones were 28 individuals at the highest applied concentration of the NS particle (300 ppm) and 22 individuals at the lower concentrations of GC particle. Emerged adults were categorized as normal and deformed moths in addition to the total adult death (those dead 48 h post emergence). The highest emergence of normal moths were recorded at the highest and lowest applied concentrations of both NS (18 moths) and GC (17 moths) particles, respectively whereas the deformed ones were fluctuated among 12 and 2 individuals at 200 ppm applied concentration of both particles. Died moths 48 h post emergence (total adult death) were only noticed at the higher applied concentrations (200 and 300 ppm for the NS particle and 300 ppm for GC). As shown in Table 2, the corrected percentages of the overall death exhibited inverse relation with the applied concentrations whereas the highest overall death percentage for the GC was fulfilled at the highest applied concentration (about 82.6% at 300 ppm) (Table 3).

The effect of the applied concentrations of both particles on larval weight gains was also addressed and graphically represented in Figure 7. In this concern, significant differences between the weight gains of both treated and check larvae were noticed (F value for the NS particle = 48.728, P = 0 and F value for the GC = 46.12, P = 0). As the 20 days old larvae weighted about 0.14 g. at the control trial, their counterparts at the treated experiments showed significant losses in their weights that ranged between 0.04 and 0.05g.

The transverse sections in untreated *G. mellonella* larvae showed the typical midgut histological layers (Figure 8a). Epithelium is the outermost layer that consists from two types of cells; elongated and columnar epithelial cells and the regenerative cells. Regenerative cells are smaller than the epithelial ones and exist at their bases. Epithelium layer is rested on the basement membrane and lined by the peritrophic membrane. The last membrane protects the epithelial cells from being contact with the food stuff (Figure 8a). Midgut tissues of the treated larvae showed several histopathological deformations (Figure 8b). The basement membrane is separated from the epithelium layer, the epithelial cells became vacuolated and lost their boundaries leaving synthetium like structure and the pretrophic membrane got detached from the epithelial cells.

4. Discussion

Although the multipurpose investments of rice chaff particles either in their normal and nano-sized forms, the assessment to clarify its insecticidal potential isn't commonly encountered and still needs much more studies.

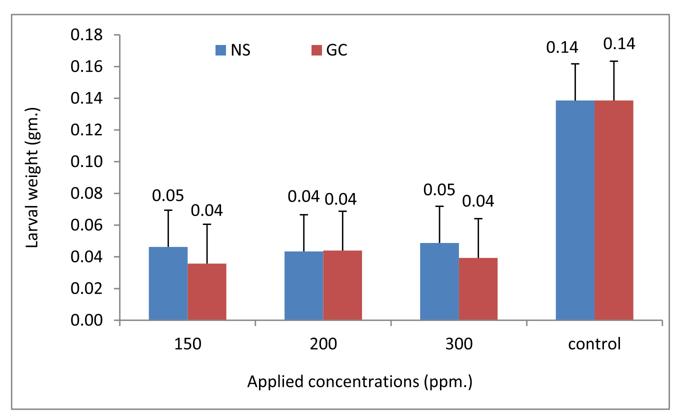

Figure 7. Effect of applied concentrations of the rice chaff particles (NS: nano-sized particle and GC: ground rice chaff particles) on the larval weight gain.

Figure 6. Malformed features, a: dead (deformed) pupae, b: larval-pupal intermediates (LP).

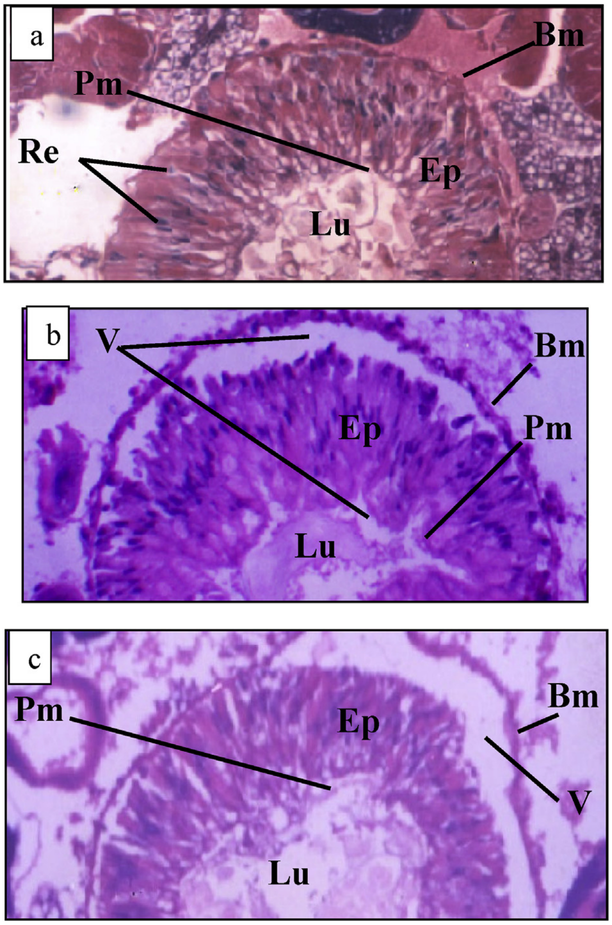

Figure 8. a: T.S. in the normal mid gut of 20 days old larvae of *Galleria mellonella*. b: T.S. in the mid gut of 20 days old larvae of *Galleria mellonella* treated with nano-sized particle (NS). c: T.S. in the mid gut of 20 days old larvae of *Galleria mellonella* treated with ground rice chaff (GC). Bm: Basement membrane. Lu: lumen. Ep: Epithelial cells. Pm: Peritrophic membrane. Re: Regenerative cells. V: Vacuoles.

The EDX spectrum of GC sample shows the highest peaks for silicon, oxygen and carbon in addition to small ones for potassium, aluminum and phosphorous. On the other hand the EDX analysis of NS exhibits only silicon and oxygen peaks confirming that the produced material is pure silica.

The extraction process of silica from rice chaff is the main factor affecting the produced silica morphology. In this manner, the XRD diffractogram of NS exhibits the presence of mixed phases of crystalline and amorphous silica as indicated by featureless diffractograms with a diffuse maxima around $2\theta = 22.2^\circ$ and a characteristic peak for crystalline silica at $2\theta = 26.7^\circ$, respectively.

Regarding surface textural analysis, GC shows a very low specific surface area (S_{BET}~3 m^2/g) and a type III adsorption isotherm according to the classification of Brunauer–Deming–Deming–Teller (BDDT). While NS showed a higher specific surface area (S_{BET} = 179.7 m^2/g) and adsorption isotherm of type II exhibiting an H3 hysteresis loop by IUPAC indicating the presence of meso-porosity (Gregg and Sing, 1982). This type of hysteresis originates from the aggregation of plate-like forms to give slit shaped pores.

From SEM image of GC, we can see the sheet-like non porous surface structure while for NS, agglomerates of nano-sized particles appear in a flake-like structure. TEM image (Figure 4c) of NS shows nano sized spheroid silica particles with size range 5–35 nm (AL-Adham et al., 2018).

The rich content of rice chaff with silica (Korotkova et al., 2016; Ummah et al., 2015) could be responsible for its induced insecticidal proprieties. The exposure of neonate *G. mellonella* larva to rice chaff rich diet may lead to the abrasion of its mouthparts by the action of its siliceous content (Massey and Hartley, 2009). The more exposure, the aggressive impacts on feeding performance, conversion of the ingested food stuff into body mass (*i.e.*, affecting the digestive ability) and obvious retardation of growth rate will be the case (Massey et al., 2006, 2007). The minor larval death 2 days post treatments with progress larval death in a time dependent manner could support the cumulative mode of insecticidal action due to the daily larval uptake of rice chaff contaminated media. This observation could decrease the likelihood of the acute toxic action (the knock-down effect) of the compounds under investigation and support their chronic death potentials. The presence of morphogenetic features (permanent larvae and LP. intermediate) could be returned to the malnutrition of the larval stages that failed to reach the critical weight required to inactivate the corpora allatum gland (the endocrine gland responsible for the production of juvenile hormone). So the juvenile hormone titer will be continuously expressed and performed an extra role in delaying the inter-molting-larval periods from the last larval instar to the obtained pupae. If such supernumerary larvae succeeded to molt despite the treatment, the molting process will be initiated then stopped due to the unbalanced state between juvenile and ecdysone (molting) hormones to produce these LP. malformed features. The retardation of the larval weight gain following rice chaff treatments could be attributed to the reorientation or diversion of its acquired energy to detoxify the toxic material instead of converting the ingested food into body mass (Massey and Hartley, 2006). As the midgut is responsible for food digestion and absorption (Sharaby and El-Bendary, 2017) in the insects, the induced histopathological deformations may be the reasonable cause for the malnutrition pattern and the developmental disorder as well (Luca et al., 2012).

The recorded LC_{50} values for both rice chaff particles revealed the insecticidal potency of the nano-sized (NS) particle more than that of the ground rice chaff (GC) one. Another interesting point was the changeable or unexpected impact of the implemented concentrations of the nano-sized (NS) particle on the larval death. *I.e.*, the lowest induced larval death at the highest applied concentration. Such point may be returned to the variation in the architecture of the prepared nano-particle that may facilitate its pathway within the larval body to its site of action to induce its effects faster than the normal sized particle. Similar impact had been found by Mekewi et al. (2012) upon bioassayed the nano zinc oxide particles. This baffling behavior needs further studies to decode its mystery.

Finally, nanotechnology will be an active point for further studies and researches during the upcoming period in terms of exploring the insecticidal potentialities and the environmental consequences in terms of their influence on the wild and marine lives, the environmental fate, the pathway of their residues…*etc*. to put the standards and commitments for the assessment of their risks and benefits before deliberation.

Declarations

Author contribution statement

Ahmed Shebl: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ahmed I. Imam: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mahmoud M. Hazem: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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