IMPACT OF RICE HUSK DERIVED SILICA NANOPARTICLES ON THE MIGRATORY LOCUST (*Locusta migratoria* - ACRIDIDAE)

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Abstract. Nanotechnology is a new trend opening up many opportunities in several fields such as agriculture and medicine. In the context of pest control and evaluation of the effect of nanomaterial on orthopteran pests, a study on the impact of silica nanoparticles on third instar larvae and adults of *Locusta migratoria (*Orthoptera: acrididae) was undertaken. Indeed, different concentration of nanomaterials was used in order to investigate the impact of this substance. On the other hand, neuro-intoxication symptoms were observed following ingestion of the grass sprayed with Silica nanoparticles; hyperexcitability separated by moments of immobility, movement disorders and intense defecation, testifying to the entomotoxic action of silica nanoparticles on the Migratory Locust. The mortality rate of treated locusts with the highest dose reached 100% after 11 days for L3 larvae and 21 days for adults. The evaluation of lethal times 50 (LT50) for this nanomaterial showed the speed action of this substance , which is 5.35 days for L3 larvae and 11.09 days in adult case for a maximum concentration of 5 mg /100 mL.

Key words: Orthoptera pest; *Locusta migratoria*; silica nanoparticles; lethal time; mortality.

INTRODUCTION

Throughout history, humanity has always been affected by locust plagues, and these plagues have always been particularly devastating for Africa. During the past centuries, this remained a recurrent theme for travelers, missionaries and naturalists, who all bore witness to the severity of the problem and its effect on food supplies in Africa [21].

Among the locusts that are enemies of Sahelian crops, the migratory locust *Locusta migratoria* (Linnaeus, 1758) is a major pest during the invasion period. This locust thus presents the phenomenon of phase polymorphism which allows it to adapt to variable ecological conditions [17]. During periods of invasions, the damage induced by of these locusts is mainly limited to grasses such as millet, corn, rice, sugar cane, wheat and other species [19]. The presence of this pest thus increases the risk of social erosion and poverty [39]. To deal with this scourge, chemical control remains the most effective until now. However, the arsenal of chemical techniques are proven to be very toxic and dangerous to the environment and with collateral effects on all ecosystems that are intolerable [15].

Owing to the increasing environmental hazards and enhanced resistance towards insecticides, along with the restrictive use of many chemical pesticides and a limited production of newer safe chemicals, has prompted active research in biological control (plant extracts and microbial culture filtrate) and in highly efficacious insecticides with novel modes of action. These are becoming increasingly important in agriculture as components of integrated pest management, resistance management strategies and to replace older classes of compounds which are perceived to carry higher safety and environmental risks [22].

The advances in science and technology in the last decades were made in several areas of insecticide usage. It includes either development of more effective and non-persistent pesticides and new ways of application, which includes controlled release formulation (CRF). The endeavors are direct towards the successful application of those compounds on crops and their efficacy and availability improvement and reduction of environmental contamination and workers exposure [32].

According to Bhattacharyyal et al. (2010) [4], the word “Nano” is developed from the Greek word meaning “dwarf”. In more technical terms, the word “nano” means 10^{-9}, or one billionth of something. For example, a virus is roughly 100 nm in size. Naturally, the word nanotechnology evolved due to use of nanometer size particles (size of 1 to 100 nm).

Nanoparticles represent a new generation of environmental remediation technologies that could provide a cost-effective solution to some of the most difficult environmental remediation problems [5]. Nanoparticles are believed to help producing new pesticides and insecticides [27]. In addition, researchers believe that nanotechnology will revolutionize agriculture, including pest management, in the near future [4]. Although, many studies have been conducted on the toxic effects of nanoparticles on bacteria, fungi and animal pathogens [10, 13, 29, 33]; a few research works have been conducted to study the toxic effect of nanoparticles on insects, particularly on pests.

Wan et al. (2005) [37] have studied the action of mixing two nanoparticles with two insecticides on the harmful mite *Epitrimerus pyri*. According to the same authors, cypermethrin and alpha-terthienyl mixed with nanoparticles of zinc oxide and copper oxide were effective against the mite tested. Yang et al. (2009) [38] demonstrated the insecticidal activity of polyethylene glycol-coated nanoparticles loaded with garlic essential oil against adult *Tribolium castaneum* insect found in stored products. In the same context, Stadler et al. (2010) [35] have shown that nanoalumina may be successfully used to control pests of stored cereals.

Merket, (2008) [23] found that targeted nanoparticles often exhibit novel characteristics like extra ordinary strength, more chemical reactivity and possess a high electrical conductivity. Thus, nanotechnology has become one of the most promising new technologies in the recent decade. Nanoparticles possess distinct physical, biological and chemical properties associated with their atomic strength.

The aim of our study is to show the impact and toxicity of silica nanoparticles against the locust *Locusta migratoria*.

MATERIALS AND METHODS

Biological material

The biological material consists of third stage larvae (L3) and adults of *Locusta migratoria*.

Breeding of *Locusta migratoria*

The study was started with 30 couples of *L. migratoria*. These locusts were collected in Adrar, a pivot located at 304 meters of altitude, 27°32'20"N latitude and 0°11'42"W longitude. The locusts were transported to the Laboratory of Dynamics and Biodiversity (L.D.B.), Biodiversity Team of Arthropods, Agronomic and Medical Interest (BAIAM) of the Department of Ecology and Environment of the University of Science and Technology Houari Boumediene (U.S.T.H.B.) where a mass breeding of this locust was undertaken. These locusts were placed in two cages (0.6 m x 0.5 m x 0.5 m x 0.3 m) depending on the stage of the study. The breeding of the locusts was maintained at a temperature of 30 ± 4°C and a relative humidity of 50 ± 5%. 80 W lamps provided continuous lighting throughout the period in the day. The diet consisted mainly of green grasse. The renewal of the food, the cleaning of the cages and containers, the humidification of the nests, as well as checking the nesting boxes for the research of ootheques were carried out daily.

Silica nanoparticles (SNP)

The SNP were provided by Professor El-Samahy and testing was undertaken in the laboratory of Plant Protection Research Institute (PPRI), Sakha Agriculture Research Station (SARS), Kafr El-Sheikh. Egypt.

The preparation of silica nanoparticles (SNP) was realised in according to Le et al. (2013) technique [20].

While silica powder at nanoscale was obtained by heat treatment of rice husk following the sol–gel method. The rice husk ash (RHA) is synthesized using rice husk with 10% HCl and 30 wt.% sulfuric acid solution, the material was burned in a muffle furnace at 600°C for 4 h to remove all incorporated hydrocarbons. The silica from RHA was extracted using sodium hydroxide solution to produce a sodium silicate solution and then precipitated by adding H_2SO_4 at pH = 4 in the mixture of water/butanol with cationic presence. In order to obtain good dispersion and uniform particle size of SNP, Surfactant (2.0 wt.%) was dissolved in the water/butanol (1:1) solvent. Subsequently, RHA-derived sodium silicate was slowly added into the CTAB/water/butanol solution, and the mixture was stirred at 60°C. Then, 0.5 mol/L sulfuric acid solution was added gradually into the suspension in order to initiate the hydrolysis-condensation reaction at pH ~ 4. The resulting gel mixture was aged at 60°C for 8 h. Then, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 wt.% of CTAB were dissolved in the water/butanol solvent with 1:1 ratio. Subsequently, RHA-derived sodium silicate was slowly added to the CTAB water/butanol solution that was being stirred at 60°C. Then, 0.5 mol/L sulfuric acid solution was added gradually into the solution in order to initiate the hydrolysis-condensation reaction. The suspension was adjusted until the pH is 4. The resulting gel mixture was aged at different temperatures in the function of time. The aged silica gel was dispersed in butanol and washed with distilled water for several times. Nanosilica was calcinated at 550°C for 4 h in atmospheric condition to remove the surfactant. The final product was obtained and stored in desiccators before further characterizations.

By transmission electron microscopy (TEM) the SNP were examined. The size distribution pattern of SNP obtained were 20 nm with a purity of 99.99% (Fig. 1).

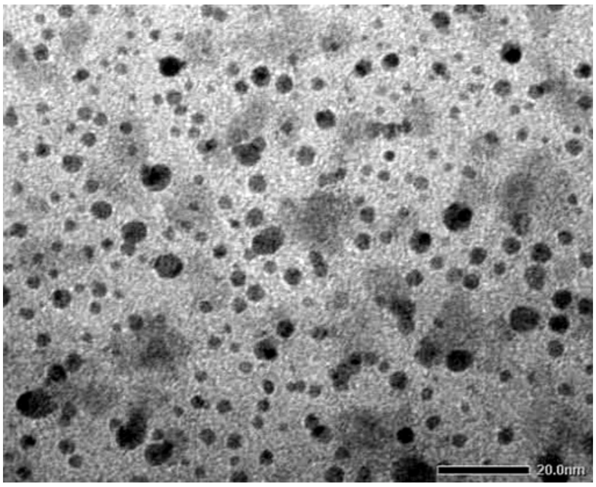


Figure 1. The shape, the size and the uniformity distibution of silica nanoparticles

Entomotoxicity study

The silica nanoparticles were diluted in distilled water (100 mL) then sprayed directly on the grass with a micro-spray. The grass treated has been given to L3 and adults of *Locusta migratoria* after 12H of fasting. The aim was to observe the actions on the mortality. For this purpose, 300 locusts divided into 10 groups, each group made of 30 individuals. These 10 groups were divided into 5 groups of L3 locusts and 5 groups of adults. Each of those two groups was made of 1 group control and 4 groups treated with 4 concentrations:

- Concentration I (D1): 0.5 mg/ 100 mL (0,5 mg of pure SNP);
- Concentration II (D2): 2 mg/ 100 mL (2mg of pure SNP);
- Concentration III (D3): 3.5 mg/ 100 mL (3,5 mg of pure SNP);
- Concentration IV (D4): 5 mg/ 100 mL (5 mg of pure SNP).

Statistical analysis

The morphology and size of silica nanoparticles were by transmission electron microscopy (TEM). The mortality data was analyzed with XLSTAT 2010 and confirmed with R Development core Team, 2017. Lethal time 50 (LT50) is calculated from the regression line of the probits corresponding to the percentage of mortality corrected for logarithms of processing time. The SCHNEIDER formula and the probit table are used:

$$MC = [M2-M1/100-M1] \times 100$$

where: MC: % of corrected mortality; M2: % mortality in the treated population; M1: % mortality in the control population.

RESULTS

Action of SNP on mortality of L3 larvae and adult of *Locusta migratoria*

Mortality studies showed that toxicity was different from the larval stage to another and from one concentration to another. Indeed, Figure 2 and table.1 show that the D4 with 5 mg / 100 mL is the fastest and most effective: locust treated with this concentration have a mortality rate of 100% after 11 days followed for L3, while in adults case was 21 days. The action of the D3 dose recorded a 100% mortality rate after 14 days for L3 larvae and 24 days for the adults. Otherwise, the reaction time of the D2 dose is the slowest with a rate of 100% after 18 days for L3 larvae, while for adults was 28 days. No mortality recorded in adults or L3 larvae individuals in batches treated with the D1 dose. Under laboratory conditions, mortality is different from that which can occur under natural conditions where larvae of different ages and adults are subjected to large thermal amplitudes, wind, rain, and other climatic factors. The daily manipulations cause injuries to different individuals of L3 larvae and adults, especially larvae which led to artificial mortality. However, we did not record any mortality for the control groups.

Lethal time 50 (LT50) of SNP on L3 larvae and adult of *Locusta migratoria*

In order to calculate the LT50 of the used nanoparticles, we have plotted the probit regression line according to the logarithm of time. The regression

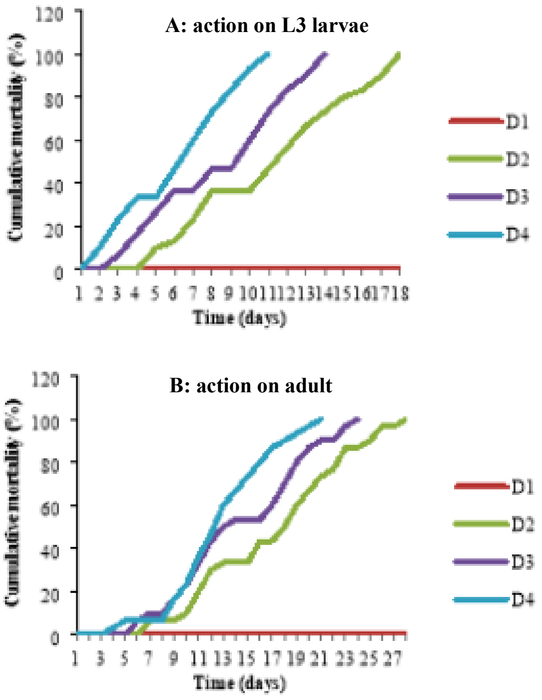


Figure 2. The action of different doses on the cumulative mortality of L3 larvae (A) and adult (B)

Table 1. Mean mortality (±S.E) of L3 larvae and adult of *Locusta migratoria* treated with different concentration of SNP

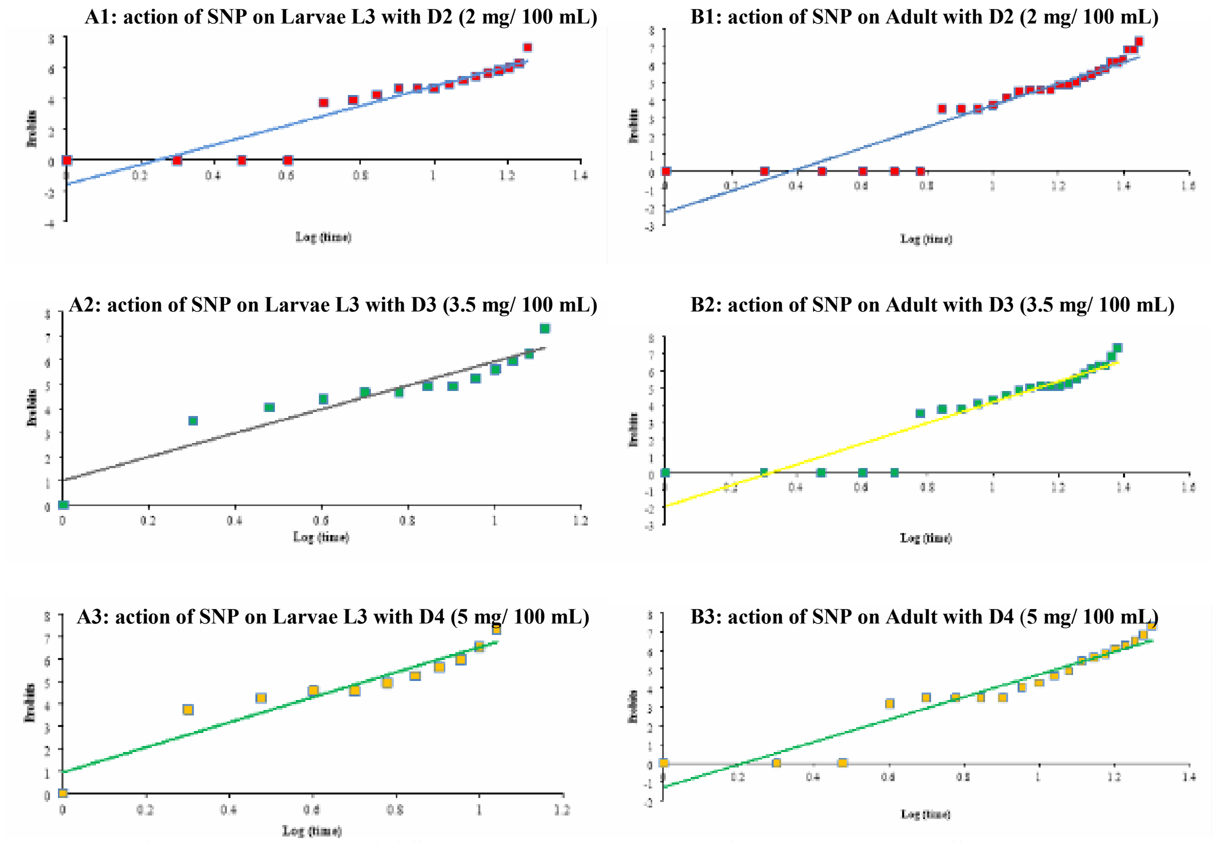
A. ADULT

Day No.	**Concentration** **D1**	**D2**	**D3**	**D4**
Day 1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Day 4	0.0±0.0	0.0±0.0	0.0±0.0	3.33±1.1
Day 5	0.0±0.0	0.0±0.0	0.0±0.0	6.66±0.92
Day 6	0.0±0.0	0.0±0.0	6.66±0.39	6.66±0.92
Day 7	0.0±0.0	6.66±0.59	9.99±0.75	6.66±0.92
Day 8	0.0±0.0	6.66±0.59	9.99±0.75	6.66±0.92
Day 9	0.0±0.0	6.66±0.59	16.65±0.19	16.66±0.58
Day 10	0.0±0.0	9.99±0.37	23.31±0.26	23.32±0.77
Day 11	0.0±0.0	19.99±0.92	33.31±0.59	36.65±0.36
Day 12	0.0±0.0	29.99±0.13	43.31±0.84	46.65±0.99
Day 13	0.0±0.0	33.32±0.843	49.97±0.46	59.98±1.23
Day 14	0.0±0.0	33.32±0.843	53.3±0.11	66.64±1.04
Day 15	0.0±0.0	33.32±0.843	53.3±0.11	73.3±0.97
Day 16	0.0±0.0	43.32±0.26	53.3±0.11	79.96±1.19
Day 17	0.0±0.0	43.32±0.26	59.96±0.98	86.62±1.91
Day 18	0.0±0.0	49.98±0.57	69.96±0.75	89.95±1.31
Day 19	0.0±0.0	59.98±0.61	79.96±1.82	93.28±0.94
Day 20	0.0±0.0	66.64±0.73	86.62±0.96	96.61±1.1
Day 21	0.0±0.0	73.3±0.15	89.95±0.81	100±0.0

B. L3 LARVAE

Day No.	**Concentration** **D1**	**D2**	**D3**	**D4**
Day 1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Day 2	0.0±0.0	0.0±0.0	0.0±0.0	10.0±0.0
Day 3	0.0±0.0	0.0±0.0	6.66±0.76	23.33±0.65
Day 4	0.0±0.0	0.0±0.0	16.66±0.58	33.33±1.07
Day 5	0.0±0.0	10.0±0.12	26.66±0.83	33.33±1.07
Day 6	0.0±0.0	13.33±0.22	36.66±0.93	46.66±0.69
Day 7	0.0±0.0	23.33±0.42	36.66±0.93	59.99±0.32
Day 8	0.0±0.0	36.66±0.81	46.66±1.62	73.32±0.63
Day 9	0.0±0.0	36.66±0.81	46.66±1.62	83.32±0.84
Day 10	0.0±0.0	36.66±0.81	59.99±1.09	93.32±1.09
Day 11	0.0±0.0	46.66±1.34	73.32±1.17	100±0.0

coefficients and LT50 values were evaluated for each concentration studied on the L3 larvae (Fig. 3 A) and adults (Fig. 3 B) of the Migratory locust. The evaluation of lethal times 50 for the different concentrations made possible to show the toxicity and rapidity of SNP. According to Table 2, the variation is depending on the stage and the dose. The TL50 evaluated for L3 larvae is shorter than that recorded for adults. We noticed a response in the L3 larvae of 10.75 days and 6.07 days for D2 and D3 concentrations respectively, while for D4, we recorded a response of 5.35 days. However, for adults, the estimated LT50 is 16.21 and 13.48 days for D2 and D3 concentrations, while it is 11.09 days for the D4.


A1: action of SNP on Larvae L3 with D2 (2 mg/ 100 mL)

B1: action of SNP on Adult with D2 (2 mg/ 100 mL)

A2: action of SNP on Larvae L3 with D3 (3.5 mg/ 100 mL)

B2: action of SNP on Adult with D3 (3.5 mg/ 100 mL)

A3: action of SNP on Larvae L3 with D4 (5 mg/ 100 mL)

B3: action of SNP on Adult with D4 (5 mg/ 100 mL)

A: Action of SNP on LARVAE L3 with different concentration (A1, A2 and A3)

B: Action of SNP on ADULT with different concentration (B1, B2 and B3)

Fig. 3. Relationship between *Locusta migratoria* and SNP according to time (A: action on larvae L3) (B: action on Adult)

Table 2. Regression equation, regression coefficient and LT50 calculated for the three concentrations of SNP

Stage	Concentration	Equations of regression lines	R2	LT50 (days)
Larvae L3	D2	y = 6.3711x – 1.5733	0.8729	10.75
	D3	y = 4.9328x + 1.0137	0.8873	6.07
	D4	y = 5.5857x + 0.9297	0.9031	5.35
Adult	D2	y = 6.0153x – 2.3088	0.8711	16.21
	D3	y = 6.1145x – 1.9641	0.8802	13.48
	D4	y = 6.0058x – 1.2767	0.9199	11.09

DISCUSSION

Action of SNP on mortality of L3 larvae and adults of *Locusta migratoria*

Very few studies have been carried out about the toxicity of nanoparticles on insects, particularly locusts, although these nanomaterial have shown their insecticidal impacts many times til now. No studies have been done til now on the effect of using silica nanoparticles on locusts, however, the use of several insecticides such as the study of Launois- Luong et al. (1988) [18] on the effect of lindane associated with lambdacyhalothrin on *Shistocerca gregaria*. Locust mortality reaches 96% after 24 hours. However, the use of insecticides remains a fast but expensive method, especially for the environment. Derbalah et al. (2012) [8] reported in their studies that SNP are more effective in pest control than other insecticides tested. In addition, silica improves the structural rigidity and plant resistance [12]. Furthermore, Debnath et al. (2012) [7] studied the cellular toxicity of nanoparticles in human fibroblast cell line and acute oral toxicity in mice. The global results in their work have shown that the nanometric of silica form is relatively non-toxic. However, future studies are required to confirm the non-toxicity. Barik et al. (2012) [2] using hydrophobic nanosilica against different mosquito species and have demonstrated that nanosilica can be applied in the control of mosquito vectors. Debnath et al. (2011) [6] showed that SNP can cause significant amount of mortality in *Sitophilus oryzae*. Otherwise, Rouhani et al. (2012) [30] successfully tested the SNP on *Callosobruchus maculatus*, a mortality rate of 100% after 14 days has been noticed. Ziaee and Ganji (2016) [40] found that the use of SNP is effective with 100% mortality in 7 days at a concentration of 200mg/kg against *Rhyzopertha domonica*. Sabbour (2013) [31] found that adults of *S. oryzae* were sensitive to SNP and caused a significant reduction in the number of eggs laid per female. They also reported that the SNP protected rice seeds from infestation with 120 days of storage.

Goswami et al. (2010) [14] studied the applications of different kind of nanoparticles viz. silver nanoparticles (SNP), aluminium oxide (ANP), zinc oxide and titanium dioxide in the control of rice weevil and grasserie disease in silkworm (Bombyx mori) caused by Sitophilus oryzae and baculovirus BmNPV (B. mori nuclear polyhedrosis virus), respectively. It was reported that hydrophilic SNP was most effective on the first day. On day 2, more than 90% mortality was obtained with SNP and ANP. After 7 days of exposure, 95 and 86 % mortality were reported with hydrophilic and hydrophobic SNP and nearly 70% of the insects were killed when the rice was treated with lipophilic SNP. However, 100% mortality was observed in case of ANP. The entomotoxic effect of silica nanoparticles were evaluated against the stored grain pest *Corcyra cephalonica* by Vani and Brindhaa (2013) [36]. Amorphous silica nanoparticles were found to be highly effective against this insect pest causing 100% mortality.

According to Smith (1969) [34], Ebeling (1971) [9], El-samahy et al. (2014) [11] and Debnath et al. (2011) [6], the absorption of SNP in the insect's cuticular lipids can be the reason of their effectiveness against various pest. It damages the protective wax (layer composed of various fatty acids and lipids that act as an effective barrier against water loss) and induces death by desiccation. This explains the appearance of several symptoms before the death of the locusts, such as balance disorders, convulsive movements, decreased appetite and consequently, a rare defecation, loss of the ability to perch on a support following the inability to perform tarsal joints, tremors of the appendages and increased respiratory rhythm are observed in our study. These same symptoms are often observed in other insects treated with SNP.

Lethal time 50 (LT50) of SNP on L3 larvae and adults of Locusta migratoria

The LT50 varies according to the dose and the stage of the insect studied. L3 larvae are more sensitive to the toxic effect of nanoparticles than adults, it is commonly accepted that larvae are more vulnerable to different toxic effects than adults, and also insect resistance increases with the stage of development, and that adults are generally more resistant than larvae [16]. In addition, the nanoparticles appear to have particularly higher toxicity than those noted for other substances. The lethal time 50 (LT50) reported for nanoparticles is lower than those noted for other toxic agents such as *Bacillus* sp. strain-B1 and *Bacillus* sp. strain-B2 in the study of Oulebsir-Mohand et al. (2016) [26] on larvae L5 of *L. migratoria* where the TL50 is 9.12 days for the highest dose. By comparing the sensitivity of *Locusta migratoria* to a local strain of Metarhizium, it's shown that the latter has a high activity with a pathogenic LT50 of 7.1 days [25]. According to Oulebsir-Mohand et al. (2016) [26] larvae treated with *B. sphaericus* have the highest LT50 with values ranging from 19.87 to 22.8 days for three used doses.

The study of the toxicity of SNP on third-stage larvae and adults of *Locusta migratoria* shows their insecticidal effect on the migratory locust. Indeed Syndromes are reported including balance disorders, convulsive movements and inability to perch on a support. These symptoms reflect the entomo-toxic effect of this nanomaterial on this locust.

This indicates that the superior entomotoxicity is due to nanosized silica itself, not due to the surface groups attached to them. This hypothesis for the physical mode of action makes the case for the use of nanocides stronger, since the insect is unlikely to become genetically selected or physiologically resistant to such a mechanism. However, the insect may develop a behavioural response to these particles by avoiding contact (Ebeling 1971) [9]. The use of such nanomaterials is more acceptable as they are safe for plants and causes less environmental pollution compared to conventional chemicals (Mewis & Ulrichs 2001 [24]; Barik et al. 2008 [3]; Rahman et al. 2009 [28]; Athanassiou et al. 2009 [1]). Moreover, application of nanoparticles on the leaf and stem surface does not alter either photosynthesis or respiration in several groups of horticultural and crop plants. Therefore, NPS and other nanomaterials may offer an important role in improving the pest management techniques for locust and other pest.

It is therefore clear that these organisms open up serious prospects for pest control, particularly during locust control campaigns, where they help to reduce the number of outbreaks. However, further studies are needed to understand the detailed mechanisms of the action of nanocides

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