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Toxicity effect of Imidacloprid and nano-Imidacloprid particles in controlling *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) under laboratory and field conditions

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Olive trees are succumbing to attack by many harmful insect species that attack fruits yield quality and quantity. Among the most important harmful olive pest species assayed in Egypt is *Bactrocera* oleae. The insecticide, Imidacloprid considered a systemic insecticide which decreases the pests infestations of many insect. The present study aimed to evaluate the efficacy of this insecticide and its nano-imidacloprid against *B*. *oleae* under laboratory and field conditions. The results obtained showed that the LC_{50} of Imidacloprid on the third larval instars was 100 mg/L. However, when nano-Imidaclorprid was applied on the target pest, the LC_{50} decreased to 83 mg/L. Under field conditions, both Imidacloprid and nano-Imidacloprid declined the rate of infestation by *B*. *oleae* with the least infestation in case of treatment with nano-Imidacloprid compared to untreated olive trees. The yield of harvested olive fruits increased due to treatment with Imidacloprid and nano-Imidacloprid with the highest yield in case of trees treated with nano-Imidacloprid.

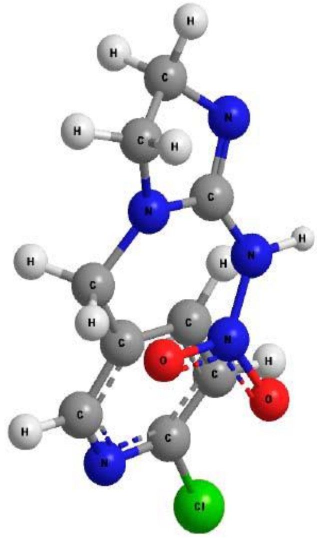
Keywords: ***Bactrocera oleae*, Imidaclorprid, nano-Imidacloprid, Toxicity,**

INTRODUCTION

Olive fruit crop considered among the very important economical crops in Egypt. The olive trees cultivated areas expanded generally in the last ten years especially in new reclaimed desert areas (Mahmoud, 2009). Olive trees are undergo, to attack by many harmful serious pests which causes a decrease on the yield quality and quantity. *B. oleae* considered among the harmful insect pests especially in Egypt, it damage most of the olive trees (Rice, 2000; Eid, 2003). *B. oleae* pest was a municipal especially in the Mediterranean countries, it found by about 98% of the olive trees planted in all world (Montiel-Bueno and Jones, 2002).

The insecticides Imidacloprid considered among the systemic insecticide which related to a class of chemicals called neonicotinoids. Specifically, Imidacloprid causes a blockage in the nicotinergic neuronal pathway which leading to the accumulation of acetylcholine, an important neurotransmitter, causing the insect's paralysis, and finally, lead to insect death. Imidacloprid is efficacious on contact and by means of stomach action. The imidacloprid insecticide which has binded much more very strongly to the neuron receptors of the insect pests than to the mammal neuron receptors, So it considered selectively more toxic to, ground application as a granular or liquid formulation, or as a pesticide-coated seed agriculture pests than mammals.

Figure.1 molecular structure of imidacloprid



Imidacloprid is exceedingly used for controlling the agricultural pests. Imidacloprid applied on plant surface , by injection of soil and trees, also on, foliar treatment. Additionally, it is applied to foundations to prevent termite damage, to control pest gardens, to treat domestic pets for flea control, and to protect trees from boring insects. The imidacloprid molecular structure as shown in figure 1.

The Nanotechnology is a promising field of interdisciplinary field research. Nanotechnology opens up a wide order of chances in many scientific fields like insecticides, medicine, pharmaceuticals, horticulture and agriculture. The possibility of uses and benefits of the nanotechnology are huge. These hue benefits include controlling of insect pests through the formulations of nanomaterials-based insecticides. Conventional strategies like integrated pest management used in agriculture are not enough, and the usage of the harmful chemical insecticides have harmful effects on animals and human beings away of the decreasing in soil fertility (Sparks et al., 2012). Therefore, nanotechnology would provide green and enough alternatives for the controlling of insect pests in agriculture without harming the nature. Ragaei and Sabry (2014) reviewed the use of nano-technology in controlling insect pests. Sabbour, (2017) use the synthetic insecticides imidacloprid against *Zeuzera pyrina* and reported that the nano imidaclopride reduce the pests infestations in the Egyptian fields. The nano *Nomuraea rilleyi, Isaria fumosoroea* and the bactira B.t (spinosad) decrease the infestations by olive pests by three fold under field conditions Sabbour, (3013a,b).

Therefore, the present study aimed to evaluate the efficacy of Imidaclorpid and nano-Imidaclorpid against *B. oleae* under laboratory and field conditions.

MATERIALS AND METHODS

The adult target insect pest *B. oleae* in these experiments were obtained from our National Research Center laboratory colony reared at 26±2°C and 60–65% RH and 12:12 h (L: D) photoperiod. Adults feed on water and a solid diet containing about 5% egg yolk. , 40% sugar and 10% hydrolyzed yeast. They were put in cylindrical glass cages (15 cm in diameter × 22 cm in height), covered with muslin, then fed on 10% honey solution.

Imidaclorpid and nano-Imidacloprid

The insecticide Imidaclorpid was obtained from company called Shanghai Fuang Agrochemical Co. Ltd (99.9% purity). The Nano-Imidacloprid was prepared by the methods used by Guan et al (2008).

The Nano encapsulation is a process which that the chemical materials release slowly and efficiently to the particular host for insect pests control. “The release mechanisms include the material dissolution, material biodegradation, the material diffusion and lastly the osmotic pressure of the material with accurate pH” (Vidhyalakshmi, et al., 2009). Encapsulated of Imidaclorpid tested nano-emulsion is prepared by a high-pressure which homogenization of 2.5% surfactant and 100% glycerol, to create stable droplets of the material which increase the retention of tested

Imidaclorprid and this causes a slow release of the nanomaterials Imidaclorprid . The release rate depends upon the protection time; consequently a decrease in release rate can increase the insect pests protection period (Nuruzzaman et al., 2016).

Laboratory experiments

Imidacloprid were prepared at six aqueous concentrations of: 2.000, 1.500, 0.750, 1.000, 0.500, 0.250, 0.125 mg/L. Olive leaves were dipped in each concentration for 10 seconds and left to dry at room temperature. The treated leaves were produced to the third larval instars (20 larvae /concentration). The control experiments (non-treated) insects were fed on olive leaves which dipped in distilled water. Each experiment was replicated five times. After seven days of treatment the percentage of mortality was recorded and then corrected against that of the control according to Abbott's formula (Abbott, 1925). Corrected mortality was calculated to according to (Finney, 1971) to estimate the LC_{50} value.

Field experiments

The field experiments were executed, at Ismailia and Ibn Malek starting from the first of July to end of August. Three random patches of olive trees were selected; each consisted of 12 trees for Imidaclorprid application, 12 trees for nano-Imidacloprid application and 12 trees for control. Both Imidaclorprid and nano-Imidacloprid were applied at the rate of 2.00 and 0.12 mg/L, respectively. Three applications were made at one week interval at the commencement of the experiment. Treatments were performed at sunset using a ten liter sprayer. Percentage of infestation/sample was calculated after 20, 50, 90 and 120 days of application. Each treatment was replicated four times. Four plots were treated with water and used as control. Random samples of olive leaves and fruits were weekly collected from each treatment and transferred to laboratory for examination. The infestation percentage of *B. oleae* was estimated in each case. After harvesting olive fruits, the yield of each treatment was weighed and expressed as Kg/Feddan.

Statistical analysis

Data were statistically analyzed by *F*-test; LSD value was estimated, using SPSS tatistical program software.

RESULTS

Table 1 shows that nano-Imidacloprid was about 1.2 times as toxic as Imidaclopirid to *B*. *oleae* third larval instars under laboratory conditions. The same findings by (Sabbour, 2015a), the LC_{50} of Imidacloprid against the olive pests, *Ceratitis capitata* and *Pryas oleae*, was 221 and 200 mg/L, respectively

Our field experiments revealed that the rate of infestation of the tested olive trees by the target insect *B*. *oleae* was significantly ($P < 0.05$) declined due to treatment with the insecticide Imidacloprid and nano-Imidacloprid compared to control insects with the least infestation in case of treatment with nano-Imidacloprid at both Ibn Malek and Ismailia regions (Table 2). The least infestation was attained after 20 and 120 days of treatment with Imidacloprid and nano-Imidacloprid, respectively. Interestingly, the infestation with the target pests decreased with the increase in time especially after treatment with the prepared nano-Imidacloprid. Similarly, Sabbour (2015a) reported that the synthetic insecticide Imidacloprid and nano-Imidacloprid reduced the rate of infestation of *C*. *capitata* and *P*. *oleae* in by three fold in olive trees. Again, Sabbour (2015b) recorded decreased infestation rate by potato tuber moth, *Phthorimaea operculella*, in plants treated with nano-fungi *Isaria fumosorosea* and *Metarhizium flavoviride*. Similar findings were also attained by Sabbour (2013) against *B*. *oleae*, *C*. *capitata* and *P*. *oleae* in olive trees treated with spinosad which decreasing the percentages of infestations in the field.

The weight of harvested olive fruits was significantly ($P < 0.05$) enhanced after treatment olive trees with Imidacloprid and nano-Imidacloprid at Ibn Malek and Ismailia compared to control trees (Table 3). Imidacloprid and nano-Imidacloprid increased the crop yield by about 131 and 159% relative to control, respectively at Ibn Malek region. At Ismailia region, such increase was about 151.3 and 198.4% relative to control in case of treatment with Imidacloprid and nano-Imidacloprid, respectively. Thus, nano-Imidacloprid increased olive crop by about 12.2 and 18.7% relative to Imidacloprid at Ibn Malek and Ismailia, respectively. Figure 2show the size of the imidacloprid by electron microscopy at 0.5 um, figure 3 show that the nano electron microscopy of nano imidacloprid at 200 nanometer.

Figures, 4 and 5 show that the infestations with *B. oleae* were decreased in both two places especially after treated with the nano imidacloprid.

Table 1. Susceptibility of *B. olea* third larval instars to Imidacloprid and nano-Imidacloprid under laboratory conditions

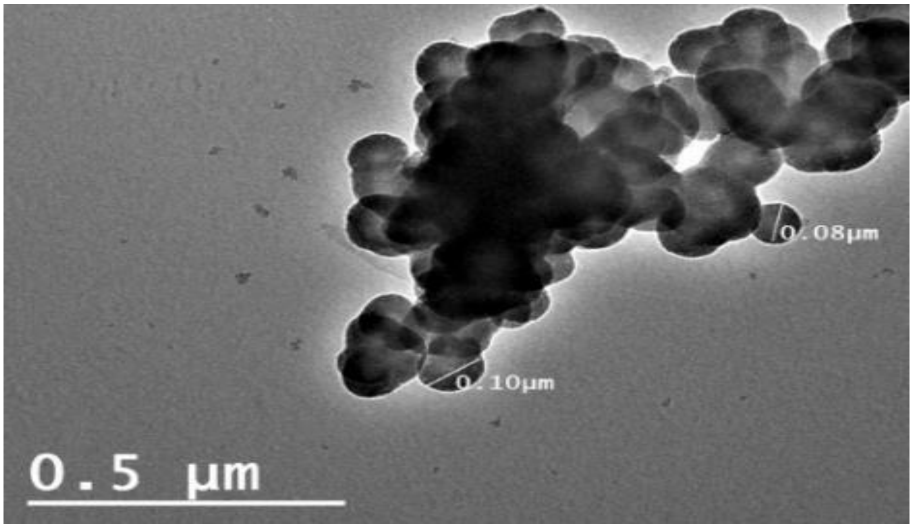
Treatment	LC_{50} (mg/L)	95% Confidence limit	Slope
Imidacloprid	100.0	77.0 - 123.0	0.01
Nano-Imidacloprid	83.0	55.0 - 101.0	0.01

Table 2. Infestation of olive trees by *B. oleae* after treatment with Imidacloprid and nano-Imidacloprid under field conditions at Ibn Malek and Ismailia regions

No. of infested plants ± SE			
Treatment	**Days after treatment**	**Ibn Malek**	**Ismailia**
Control	20	22.1 ± 5.7	22.1 ± 2.5
	50	71.1 ± 2.3	75.0 ± 2.2
	90	90.0 ± 4.4	95.0 ± 3.6
	120	99.0 ± 1.2	99.0 ± 1.2
Imidacloprid	20	12.1 ± 5.1	12.1 ± 2.5
	50	21.1 ± 2.3	25.0 ± 2.2
	90	40.0 ± 4.4	45.0 ± 3.6
	120	79.0 ± 1.2	81.0 ± 1.2
Nano-Imidacloprid	20	10.1 ± 5.1	10.1 ± 2.5
	50	10.0 ± 2.3	10.0 ± 2.2
	90	9.0 ± 4.4	10.0 ± 3.6
	120	7.0 ± 7.2	8.0 ± 6.2
***F*-test**		33.1	30.4
LSD (P = .05)		80.0	77.0

Table 3. Weight of harvested olive fruits treated with Imidacloprid and nano-Imidacloprid and infested by *B. oleae* at Ibn Malek and Ismailia regions

Weight of yield (Kg/Feddan)		
Treatment	**Ibn Malek**	**Ismailia**
Control	2120.0 ± 20.72[b]	2009.0 ± 81.50[b]
Imidacloprid	4896.0 ± 20.11[a]	5094.0 ± 31.18[a]
Nano-Imidacloprid	5491.0 ± 70.10[a]	5994.0 ± 51.10[a]
***F*-test**	40.1	39.4
LSD (P = 0.05)	89	87



. **Figure 2. Scanning by electron microscopy for imidacloprid at 0.5 um.**

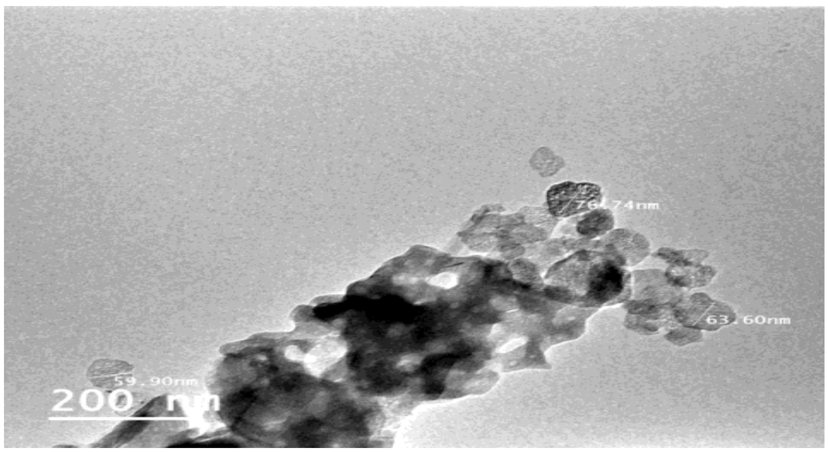


Figure 3. Scanning by electron microscopy for nano-imidaclorprid at 200 nanometer

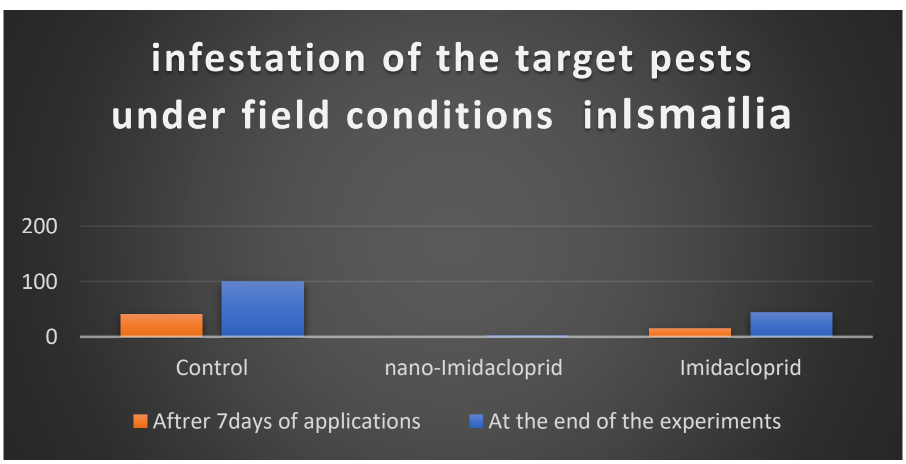


Figure 4. Effect of imidacloprid and its nano tested against *two target pests* under field conditions in Ismailia.

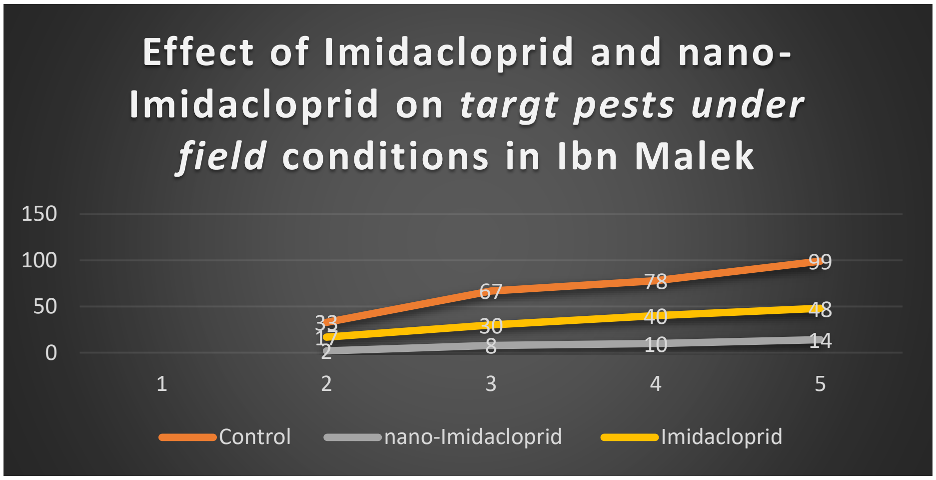


Figure 5. Effect of imidacloprid and its nano tested against *two target pests* under field conditions in Ibn Malek.

These results are in consistence with those obtained by Sabbour (2015a) for olive trees treated with Imidacloprid and nano-Imidacloprid and infested by *C*. *capitata* and *P*. *oleae*. Also, treatment of potato plants, infested by *P*. *operculella*, with nano-fungi *I*. *fumosorosea* and *M*. *flavoviride* increased the yield (Sabbour, 2015b). Similar results were obtained by Sabbour (2013) for spinosad-treated olive trees that were infested by *B*. *oleae*, *C*. *capitata* and *P*. *oleae*. Sabbour 2017 , found that the olive weight increased after bioinscticid applications in the field. Sabbour and Nayera Solieman 2017, reported that nano-biopesticides application increase the productivity of the olive fruits in the trees tested under field conditions. Sabbour, (2008) found that, the microbial control agent bacteria *Bacillus thuringiensis* control and reduce the infestations by *Prey. oleae* in olive fruits in olive trees. The same obtains recoded by Sabbour and Abd _El –Rahman 2012). Our results agree with Sabbour and Shadia and Shadia E-Abd-El-Aziz (2010) who could to apply some bioinsecticides *P. fumosoroseus* which combined with mustard oil against *Bruchidius incarnates* which detected that they highly toxic aginst the target inect peststested.

Numerous studies have demonstrated that the heterogeneous photocatalysis of pesticides is an effective process to degrade pesticides and to mineralize some of them Burrows et al., (2002) and Konstantinou and Albanis (2003).

To enhance the solubility of IMI and to maintain continuous sink conditions throughout the study period, 1% (w/v) SLS was used with phosphate buffered saline (pH 7.4) in the receptor compartment (represents systemic component) Guan et al. (2008). They reported that, when the multilayers are exposed to different environment conditions (with respect to pH, ionic strength, etc.) then the PE multilayers undergo molecular rearrangements that may increase or reduce defects. There are two possibilities for the significant change in the release .

A large specific surface area leads to a fast adsorption to the reactants and enrich enough nano-IMI in contact with the catalytic activity sites of TiO_2.

El-bendary1 and El-Helaly (2013) indicated that nano-silica seems to enhance tomato plants in controlling the cotton leaf worm, Spodoptera littoralis; this control tactic increased the obtained yield. Similar results were recorded when used potassium silicate for both the second and third larval instars, as the percentage reductions ranged 41.61-51.50 and 22.07- 36.68 %, respectively,and reduction in feeding consumption of the fourth instars ranged 15.56 - 31.50 %. Goussain et al. (2002) registered increased mortality and cannibalism in groups of fall armyworm, Spodoptera frugiperda for the larvae of the second and sixth instars with silicon application. Results of Goussain et al. (2002) proved that *Spodoptera frugiperda* larvae displayedincreased mortality, cannibalism and mandibular wear after feeding on corn plants fertilized with silica. Similar results were obtained by Massey et al., (2006) who indicated that increasing silica content of grasses deterred feeding and reduced the growth rates and feeding efficiency of *Spodoptera* sp

CONCLUSION

nano- Imidacloprid was more effective than Imidacloprid only in controlling *B. oleae*. These results encourage the extension in the use of nanotechnology for harmful olive insect pest control.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest".

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

Prof. Dr. Sabbour, M. M.and Prof. Dr. El-Sayed H. Shaurub, share together in putting the idea of the research, they designed together all the laboratory and field experiments, share in the statistical analysis by the SPSS program and writing the research paper also, reviewed the manuscript. Both authors read and approved the final version of the research.

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