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Comparison between the toxicity of nano and bulk formulations of imidacloprid against wheat aphid, bird cherry-oat aphid, *Rhopalosiphum padi* L

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Abstract

Recently, nano-insecticide formulations and new insecticide application methods are promised in pest control improvement. Wheat crop is infested by many sucking pests, such as cereal aphids in Upper Egypt. The toxicity of the recommended dose of imidacloprid (70% WS GAUCHO®) as positive control, the full and half recommended dose of nano and bulk imidacloprid 25% WP (BEST®) formulations were investigated under laboratory and field conditions against bird cherry-oat aphid, *Rhopalosiphum padi* L. The leaf-dip bioassay results indicated that the nano-imidacloprid formulations had higher potency ratio than the bulk formulations by 2.53 to 7.17 folds. Significant reduction percentage of aphids was recorded in seed treatments of field wheat crops, the aphid reductions reached 95% after 60 days. All insecticide treatments showed significant increasing in grain yield ranging from 10.55% to 22.02% higher than the control. The results concluded that the nano-imidacloprid formulation is promising formulation in cereal aphid control. Moreover, the seed-treatment is better than foliar treatment as a pest control tool for protecting the wheat seedling against sucking insect pests.

Keywords: nano- insecticides, toxicity, seed treatments, grain yield.

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1. Introduction

Wheat, *Triticum aestivum* L. (Family: Poaceae) is one of the most important food crops for human and animal feed. The Egyptian cultivated area of the wheat crop is around 3,258969 million feddans, producing about 9 million tons in Egypt (FAO, 2016). Wheat crop is attacked by many insect pests that cause great damages and reductions in the yield**.** Five cereal aphid species (*Rhopalosiphum padi, Schizaphis graminum, Rhopalosiphum maidis, Sitobin avenae, Metopolophium dirhodum* are the most serious key insect pests attacking wheat in Upper Egypt**.** (Abd-Ella, 2016; Abdel-Rahman, 1997). In addition to, the direct damages of aphids by producing honeydew that encouraging the growth of softy moulds, which considerably interface with the host photosynthesis process by inserting their stylet on plant cells and tissues, triggering reactions and cell death by the toxins and enzymes in their saliva, and by sucking plant sap. *Rhopalosiphum padi* causes indirect damages by transmission of Barley/Cereal Yellow Dwarf Virus (B/CYDVs) the causal agents of Yellow Dwarf Disease (YDD) (Parizoto et al., 2013). Insecticides are a widely traditional method used for controlling the cereal aphids. Foliar application of broad-spectrum insecticides may reduce natural enemies(Galvan *et al.*, 2005),contributing to pest resurgence. Seed treatment, another application method, with neonicotinoids has advantages especially the long protection time when compared to certain application of pesticides, particularly when used to control insect pests that feed on seeds or seedlings early in the season. Moreover, the neonicotinoids have a combination of high toxicity to insects and low toxicity to mammals that make them safer than many of the older insecticides. Seed application allows the amount applied per area to be greatly reduced compared to soil or foliar applications (Nuyttens et al., 2013; Elbert et al., 2008). Imidacloprid is belong to neonicotinoid group of chemicals which act as systemic insecticide against sucking pests. The neonicotinoids have quick knockdown effect on target pest by interfering with transmission of impulse in the nerve system. The quick and excellent systemic and translaminar activity of these insecticides make them able to control sucking pests such as aphids, whiteflies and other virus transmission insects. Neonicotinoid application reduces infection rate and spread of many crop viruses (Bethke *et al.*, 2001; Elzen, 2001; Elbert *et al.*, 1998; Westwood *et al.*, 1998; Dewar, 1992; Knaust and Poehling, 1992). Due to the side effects of conventional insecticide, the development of the insecticide application and insecticide formulation are valuable trend to alleviate these bad effects. Nanotechnology can introduce promised nano-insecticides that have so different physicochemical prosperities. Few studies are available regarding the toxicity of these nano-insecticides on insects. The objective of the present study was taken to compare between the toxicity of nano and bulk formulations of imidacloprid against bird cherry-oat aphid, *R. padi* under field and laboratory conditions. Moreover, the effect of the seed treatments of the tested insecticides on grain yield crop.

2. Materials and methods

2.1 Insecticides nano preparation

Two insecticide imidacloprid formulations (Best 25% WP, EL-Helb Pesticides & Chemicals, Egypt and imidacloprid Gaucho 70% WS, Bayer Crop Science, Egypt) were supplied from the Central Pesticide Laboratory, Egypt. The insecticide powders were milled in a FRITSCH PULVERISETTE **2** ball mill for 1 hour to obtain the micro-size formulations. X-ray diffraction (XRD) of the prepared nano formulations were recorded using PW 1700 X-ray diffraction with Cu_{α} K radiation (λ = 1.5406 angstrom) in diffraction angle (2 θ) range from 20^{o} to 60^{o} with step 0.06^{o}/min. Figures (1, 2) represents X-ray diffraction pattern of the treated powder. The line broadening of the XRD peaks indicates that the prepared material consists of particles in the nano-size range. The pesticide particle size was calculated from–Scherrer' equation:

Where, $D = 0.89\ \lambda / \beta \cos \theta$........(1)

(0.89 is Scherrer's constant, λ is the wavelength of X-rays,(θ)is the Bragg diffraction angle, and(β) is the full width at half-maximum (FWHM) of the diffraction peak .The average particle size of was found to be 21.8 nm for best and 18.1nm for Gaucho.

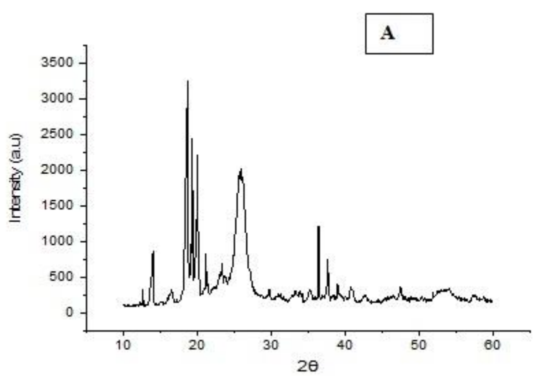

Figure 1: X-rays diffraction of treated insecticide imidacloprid 25% WP.

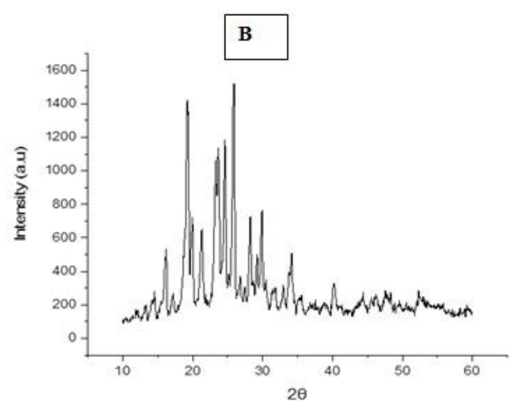

Figure 2: X-rays diffraction of treated insecticide imidacloprid 70% WS.

2.2 Insecticide laboratory bioassay tests

The relative effectiveness of nano and bulk formulations of imidacloprid neonicotinoid insecticide was tested under laboratory conditions.

2.3 Test insect

The field population of the oat aphid, *Rhopalosiphum padi* was collected from infested field wheat batches under Assiut prevailing conditions. The apterous aphids used in this study were of approximately the same size and weight for all the toxicity tests. Fresh infested wheat samples were collected by cutting plants and transferred alive to laboratory on their host plant leaves in plastic bags immediately before assays where mean temperature reached about $26 \pm 2^{\circ}$ C. The leaf dip-bioassay technique (O'brien et al., 1992) was followed to evaluate the toxicity of insecticides. Five to six concentrations of each insecticide plus commercial surfactant (frotop®) were prepared. About 10 to 15 adults, approximately of same size, were dipped for five seconds per concentration. The treated aphids could dry at room temperature for about half an hour. Control batches of aphids were similarly dipped in a solution of water plus (frotop®). After the treated batches of aphids had dried they were individually transferred to petri dishes (9 cm diameter) and held for 26 ± 2Cº, 60 ± 5 RH% and photoperiod 12:12 (L:D). Aphid mortality was recorded after 12, 24 and 48 h for treatments with the aid of a binocular microscope. An aphid was considered dead if it was in cap-able of coordinated forward movement and changing of color body to black. The toxicity experiment of each compound was repeated twice, and the results were corrected by Abbott's formula (Abbott, 1925). Median lethal concentration (LC_{50}) and slope evaluation of the tested insecticides were determined by Probit regression analysis program.

2.4 Preparation of imidacloprid formulations as seed treatment

Seeds of the wheat variety Sids (12) were put in a plastic container. Each insecticide formulation was mixed with the seeds at rate of 9.8 g a.i kg^{-1} seeds, it is equal the recommended rate by imidacloprid (Best®25%WP), and 3.5 g a.i. kg^{-1} seeds (it is equal the recommended rate by imidacloprid (Gaucho® 7 g kg-1 seeds) as manufacture label. Gaucho®, the only neonicotinoid insecticide recommended as seed treatments in certain crops. The control seeds were treated with water. Carefully shucked in then the seeds were gently participated on the desk left in air to dry in shade.

2.5 Field experiment and data collection

A field study was conducted to compare the efficacy and persistence of the nano-imidacloprid formulations with conventional (Bulk) formulations against cereal aphids. On 10th December, 2018, the treated seeds of wheat variety Sids (12) were cultivated in plots at the Experimental Farm of Faculty of Agriculture, Assiut university, Egypt. The area of each plot was 1/400 feddan (0.42 hectare). A randomized complete block design (RCBD) with 3 replicates to every treatment and 3 untreated replicates as control was used. After 22 days from planting, the aphid appeared on the wheat plants, the wheat aphid, *R. padi* began to occupy on the upper and lower leaves of wheat plants. The number of wheat aphid on 5 randomly selected wheat tillers from five locations per plot was recorded. The wheat aphid populations were weekly recorded until the maturity of the wheat plant. The aphid species identification was done using binocular in the Insect Laboratory, Plant Protection, Faculty of Agriculture, Assiut University, Egypt. The percentage (%) reduction of insect infestation was calculated according to a modified version of Abbott's formula (Abbott, 1925):

$$\text{Reduction (\%)} = 1 - \frac{Tn}{\text{Cn}} \times 100$$

Where; T = treatment, C = control, N= the number of aphids.

Grain yield: Average yield of grain wheat (kg /feddan) (feddan = 0.420

hectares = 1.037 acres) was assessed using the harvest from the treated plots and the control of wheat crops.

$$\text{Percent increase in yield over the control} = \frac{\text{Yield in treatment} - \text{Yield in the control}}{\text{Yield in the control}} \times 100$$

3. Results and Discussion

The toxicity of two bulk-imidacloprid formulations (Best® and Gaucho®) and their prepared nano-formulations were investigated against the apterous adults of *R. Padi* using the leaf-dip bioassay under laboratory conditions. Based on the LC_{50} values and the overlapping at 95% confidence limits represented in Tables (1 & 2), data indicated that most of bulk and nano imidacloprid formulations (WS Gaucho ®) showed significant higher toxicity against wheat aphids than the analogous formulations of imidacloprid (WP Best®) after 12, 24 hours of the treatment, where, the LC_{50s} values of Gaucho® bulk and Best® bulk formulations were 1406.78, 4018.83; 608.92, 1584.23 µg a.i./ml after 12, 24 hours, respectively. Whereas, the opposite is true after 48 hours post treatment, the Best® formulations have the higher toxicity effect than the Gaucho® formulations. In general, the toxicity of all imidacloprid formulations is dependently increased with the increased time after treatment.

Table 1: Comparison the toxicity of bulk and nano-Imidacloprid neonicotinoid insecticide (BEST®) against the adult apterous of bird cherry-oat aphid, *Rhopalosiphum padi* after 12, 24,48 h post treatment using the leaf-dip bioassay.

Time (Hour)	Bulk-Imidacloprid 25%WP(BEST®)				Nano-Imidacloprid 25%WP(BEST®)				Potency Ratio
	LC_{50} (µg a.i./ml) (Confidence Limits 95%)	Slope ±SE	χ2	P	LC_{50} (µg a.i./ml) (Confidence Limits 95%)	Slope±SE	χ^2	P	
12	4018.83 (2327.29-10095.46)	1.09 ±0.10	9.64	0.140	1585.70 (1289.94-2028.84)	1.45 ±0.11	6.19	0.402	2.53
24	1584.23 (959.55-3455.33)	1.03 ±0.08	14.55	0.024	253.43 (126.80-501.66)	0.54 ±0.06	10.72	0.097	6.25
48	81.63 (53.67-114.15)	0.68 ±0.07	3.08	0.799	7.470 (1.91-16.72)	0.53 ±0.07	1.97	0.922	11

Potency ratios:LC_{50} of Bulk-Imidacloprid ⁄ LC_{50} of Nano-Imidacloprid; Chi-square = χ^2 value.

Table 2: Comparison the toxicity of bulk and nano-Imidacloprid neonicotinoid insecticide (GAUCHO®) against the adult apterous of bird cherry-oat aphid, *Rhopalosiphum padi* after 12, 24, 48 h post treatment using the leaf-dip bioassay.

Time (Hour)	Bulk-Imidacloprid 70%WS (GAUCHO®)				Nano-Imidacloprid 70%WS (GAUCHO®)				Potency Ratio
	LC_{50} (µg a.i./ml) (Confidence Limits 95%)	Slope ±SE	χ2	P	LC_{50} (µg a.i./ml) (Confidence Limits 95%)	Slope±SE	χ^2	P	
12	1406.78 (788.20-3398.83)	0.61 ±0.07	6.07	0.415	271.45 (200.45-392.59)	0.76 ±0.07	4.74	0.577	5.18
24	608.92 (316.86-1843.73)	0.39 ±0.06	2.49	0.869	129.57 (91.44-190.88)	0.59 ±0.06	7.10	0.311	5
48	132.34 (77.69-250.36)	0.38 ±0.06	3.47	0.748	18.44 (10.05-28.51)	0.56 ±0.07	4.93	0.552	7.17

Potency ratios: LC_{50} of Bulk- Imidacloprid ⁄ LC_{50} of Nano- Imidacloprid, Chi-square = χ^2 value.

The toxicity data (Tables 1 & 2) showed that the nano formulation of imidacloprid (Best®) had significantly higher potency effect than its bulk formulation by 2.53, 6.25 and 11 folds respectively, after 12 h, 24 h, and 48 h post treatment. Also, the nano formulation of imidacloprid (Gaucho®) had significant higher potency than its bulk formulation by 5.18, 5 and 7.17 folds. Iimidacloprid and thiamethoxam had a better efficiency against wheat aphids than acetamiprid and dinotefuran and caused an increase in wheat production (Abd-Ella, 2016). In the recent years, nano particles have received much attention for controlling as agriculture pathogens. The quick and increased the toxicity of imidacloprid after time post treatment against wheat aphids could be explained imidacloprid is one of the neonicotinoid insecticide group. Neonicotinoids act as neurotoxic effect of on nicotinic acetylcholine receptors (nAchR) (Tomizawa and Casida, 2005)**.** In the present results, the variation in potency effects of the Best® and Gaucho® formulations depends on several factors such as the insecticide formulations, the particle size, penetration of the plant, and the prevailing environmental conditions during experimental seasons. All these factors can play a role on the pesticide disappearance among plants and influencing the efficiency of the tested insecticide. In the other hand nano-formulation increases the efficiency of insecticides and also reduces the dose level required to pest control work. In addition, pesticide permeability during the layers outside the surface of the plant depends on the amount of pesticides used in the plant tissues, and on the rate of demolition, which also varies greatly depending on the chemical composition of the compound tested under the field conditions (Saad et al., 2019; Farha et al., 2016). Little research that had been carried out to investigate the toxicity effect of nanoparticles on insects.(Guan et al., 2008) showed that toxicity of the bulk formulation of imidacloprid increased when coated with nano particles. Our results are supported by Assemi et al. (2014) who found that the LC_{50} values for nano imidacloprid, were 37.919, 69.623 µl/ ml, respectively after 72 hours. Their results showed that nano-pesticides are more effective and toxic to insect pests, compared to conventional insecticides. On the other hand, (Rouhani et al., 2012) were evaluated the insecticidal activity of silver nanoparticles against the *Aphis nerii* . They found that Ag nanoparticles can be used as a valuable tool in pest management programs of *A. nerii*. Our laboratory results are promised; these results need more investigation under the field conditions. It is of interest to point herein that the nano-neonicotioid insecticides showed great may be reducing effect on *Liriomyza trifolii* (Burgess) tested than their bulk formulation tested insecticides(Saad et al., 2019). The manufacture recommended dose of imidacloprid formulation (1X) and the half dose (0.5 X) of bulk and nano of Best® and Gaucho® formulations evaluated as a seed treatment for reducing and delaying the *R. padi* infestation of wheat plants in the field. The aphid's infestation on wheat crop started after 60 days, of sowing. The aphid population was observed during vegetative stage and continued up to last week of February. The the bulk and nano formulations of imidacloprid in all treatment showed significant lowest aphid population as compared to untreated (Control) after 60, 74 and 102 days of sowing. In significant difference has occurred in reduction % between the insecticide treatments. Also, the half dose of nano and bulk imidacloprid formulations gave positive results in reducing % (reaching 95%) of aphids on wheat crops (Figures 3 & 4).

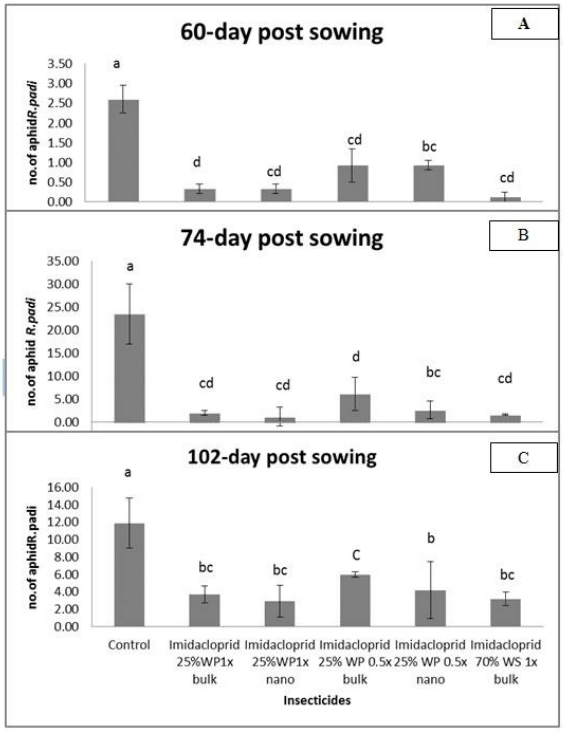

Figure 3: The mean of aphids/tiller at 60(A), 74(B), and 102(C) days of sowing. The error bars indicate the standard deviation of means. The figures with the same letter showed insignificant differences at 5% probability level, LSD test.

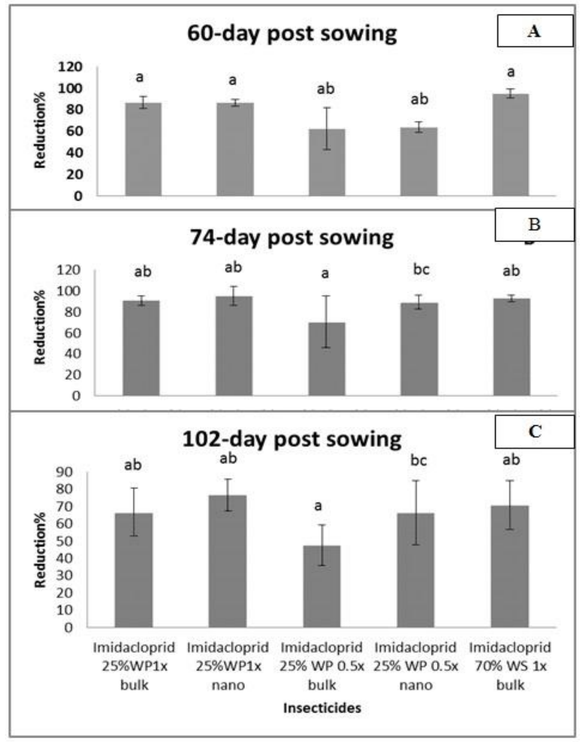

Figure 4: The mean reduction percentage of aphids at 60(A), 74(B), and 102(C) days of sowing. The error bars indicate the standard deviation of means. The figures with the same letter showed insignificant different from each other at 5% probability level, LSD test.

Burd et al. (1996) reported acute toxicity to aphids fed on plants grown from seeds treated with imidacloprid, protected plants for 45 days after sowing. While, imidacloprid in combination with tebuconazole can control aphids for 8 weeks when applied as a seed treatment by Ahmed et al. (2001). Our results are also in agreement with the results Suhail et al. (2013) who found that aphid population was lowest on plants of wheat which were grown with seed treated with Hombre and Actara. Our results are also in agreement with those of Royer et al. (2005) who found that the seed treatment with imidacloprid and other insecticides decreased the population of sucking insect pests such as cereal aphids and leaf hoppers (Khan et al., 2012; Zeb et al., 2011; Aheer et al., 2006; Aslam et al., 2004). Several investigators demonstrated that the aphid infestation on wheat crop started in the first week January 60 days after sowing. A gradual increase in aphid population was observed during vegetative stage and continued up to last week of February. On the other hand, the higher yield was observed in plots treated with imdacloprid + tebuconazole followed by dinotefuran, matrine, and pymetrozine respectively by (Ali et al., 2018). The results of grain yield are influenced by different insecticide treatments (Figure 5) where the grain yield production per feddan treated with Bulk imidacloprid Best® (1X), Nano imidacloprid Best® (1X), Bulk imidacloprid Best® (0.5X), Nano imidacloprid Best® (0.5X), and imidacloprid Gaucho® (1X) was 3204, 3236, 3192, 2932, and 2932 kg/feddan, respectively compared with the control (2652 kg/feddan). Subsequently, yield increasing as compared to untreated (Control) percentage was 20.81, 22.02, 20.51, 21.11, and 10.55 %, respectively.

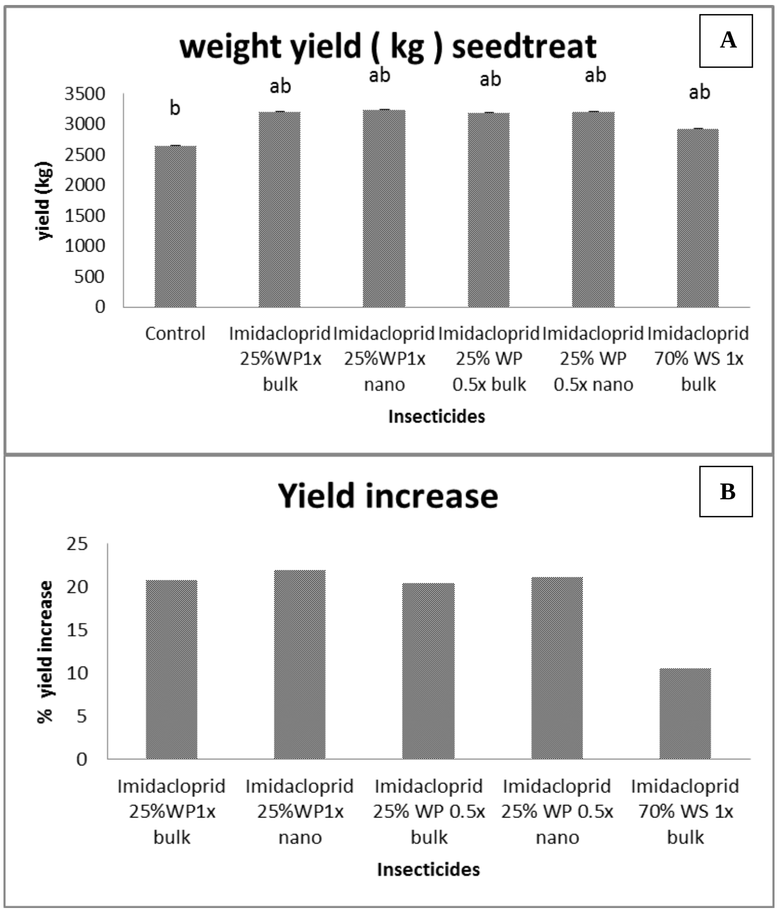

Figure 5: Estimated average seed yield of grain wheat (3A) and increase percentage of yield(3B) due to imidacloprid neonicotinoid insecticide formulations seed treatments compared to the control. The error bars indicate the standard deviation of means. The figures with the same letter showed insignificant differences at 5% probability level, LSD test.

In a similar study, the imidacloprid seed treatment enhanced the grain yield when used against Russian wheat aphids Moreover, in our results the half dose of nano and bulk imidacloprid formulations showed positive results in grain yield of wheat crops (Tolmay et al., 1997). The results of the present study are in accordance with the findings of (Royer et al., 2005) who achieved 100% morality of aphids through some botanical insecticides, this may be due to difference in crop and environmental factors as they tested on wheat plants. In addition imidacloprid was most effective with maximum mortality 97% and pymetrzine 87%, respectively after 14 days of application (Shafiq et al., 2015). VoSTřel (2010) found 100% mortality in resistant population of wheat aphids when treated with imidacloprid. Another study on effectiveness of imidacloprid and other insecticides indicated that imidacloprid-treated oat or wheat plants reduced adult longevity and fecundity of three cereal aphid species as compared to non-treated plants (Gray et al., 1996). Imidacloprid 200 SL at ml/ha treatment was most effective against wheat aphid *Sitobion avenae* , *Rhopalosiphum maidis* (Joshi and Sharma, 2009). Our finding are supported by other publications especially on faba bean seed treatments (Abdu-Allah and Hashem, 2017) who found that imidacloprid and thiamethoxam and acetamiprid as cowpea seed treatment significantly increased the cowpea height growth compared to untreated control. However, the longevity, fertility and population increase of *S. avenae* feeding on wheat plants treated with sublethal concentrations (LC_{10}) of imidacloprid was not greatly affected, and the longevity, fertility and population increase of *S. avenae* reduced significantly at the median lethal concentration (LC_{50}) of the four neonicotino (Miao et al., 2014). Lower fecundity has also been observed in *Aphis gossypii* after treatment with imidacloprid (Gerami et al., 2005). Also, imidacloprid was effective in reducing the population of aphids on brinjal (auberggine) and in increasing the seedlings total leaf chlorophyll over those of untreated plants (Jarande and Dethe, 1994). The present results are in same line with those reported by (Abdu-Allah, 2012), who studied the toxicity of imidacloprid on cowpea aphid, *A. craccivora* and *B. brassicae*, after 48 h using leaf-dip bioassay technique. He stated that imidacloprid was the least toxic compound against *A. craccivora* with LC_{50} of 2.04 mg/L and against *B. brassicae* with LC_{50} of 2.14 mg/L.

4. Conclusions

Our results concluded that the nano formulation of imidacloprid was more potency effective and toxic to wheat aphids, *R.padi*, compared to conventional insecticides. These studies clearly showed the need to research and development under the field conditions. Also the half dose of nano and bulk imidacloprid formulations give positive results in reducing percentage of aphid and its increasing yield percentage by about 22.02%. Seed treatment provides excellent control at early stage of infestation by aphid, good plant vigor and thus greater yield and less residues with long plant protection for 42 days after sowing. Conversely, their management in the later stage of infestation (foliar treatment).

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