Role of imidacloprid and chlorfenapyr nanocapsule in control of hunting billbug, Sphenophorus venatus and white grub, Phyllophaga crinita as principal pests of golf courses

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Abstract

Both hunting billbug, *Sphenophorus venatus* and white grub, *Phyllophaga crinita* are considered the most destructive pest for golf courses not only in Egypt but worldwide. This work is considered the first record of *P. crinita* in Egypt. So, many efforts were carried out to control these pests. In this work nanopesticides formulations were used against these pests; imidacloprid nanocapsule and chlorfenapyr nanocapsule under laboratory conditions. Three concentrations were used for each nanocapsule. Two stages for each pest were treated; the third and sixth instar larvae of *S. venatus* and the first and third instar larvae of *P. crinita*. The obtained results showed that the nanocapsule formulations of both imidacloprid and chlorfenapyr were very effective against *S. venatus* and *P. crinita* larvae. The LC_{50} of imidacloprid and chlorfenapyr nanocapsule against the sixth instar larvae of *S. venatus* were 10.3 and 11.8 ppm, respectively, and 8.8 and 9.1 ppm, respectively, against the third instar larvae. The corresponding results with the third and first instar larvae of *P. crinita* were 11.2 and 17.2 ppm, and 8.7 and 11.6 ppm, respectively. The results showed also imidacloprid nanocapsule was more effective than chlorfenapyr nanocapsule especially, with the first concentration; the percentages of mortality ranged between 91.7 to 96.7% with imidacloprid nanocapsule compared with 75 to 88.3% with chlorfenapyr nanocapsule. The obtained results found that the nanocapsule formulations were very promising in the control of *S. venatus* and *P. crinita* larvae. These formulations can be reduced soil contamination compared with the traditional formulations.

INTRODUCTION

Golf courses suffer from many pests, especially during the spring season. This damage of course affects the quality of golf courses. The hunting billbug, *Sphenophorus venatus* (Buss and Huang 2009) and white grub, *Phyllophaga crinita* (Vittum et al., 1999) are considered the most destructive insect pests on golf courses. *S. venatus* is a small weevil that can cause several damages to grass stems, stolons, and rhizomes of turfgrass on golf courses. The adults of *S. venatus* make pores in the soil or in the grass steam and lay the eggs in it. The eggs hatch after seven days and neonate larvae have white creamy color. The larval stage has six instar larvae. The sixth instar larvae drop into the soil and feed on roots. The pupa (pupae begin off-white in color, darken to a rusty brown) stay under the soil and the adults are most active on the soil surface at night (Huang and Buss, 2009). In Egypt, *S. venatus* the first recorded by El-Maghraby et al. (2022). The turf grass infested with *P. crinita* suffered from wilting, chlorosis, and a reduction in sod strength (Graf et al., 2017). If the *P. crinita* infestation was increased up of economic threshold this pest can remove the entire root system, resulting in the appearance of dead patches (Vittum et al., 1999).

Therefore, many traditional practices were used to overcome these pests, such as biological and chemical control (Hesselsøe et al. 2022). In some countries such as Germany, the entomopathogenic nematodes (EPNs) *Heterorhabditis megidis* and *H. bacteriophora* were applied successfully on golf course turf against *Phyllopertha horticola* and *Aphodius contaminatus* grubs. (Sulistyanto and Ehlers R-U.,1996). In Norway, the (EPNs), *H. bacteriophora*, were used against golf course pests (Hofsvang and Sundbye, 2020). In 2003, Vestergaard found that application of the entomopathogenic fungi, *Metarhizium anisopliae* against the chafer grubs gave promising control. In England, chlorantraniliprole was used against the chafer grub larvae (Syngenta, 2022).

Using traditional pesticide formulations has many side effects on the environment. Rational formulations such as nanoformulations can balance the efficacies of pesticides and reduce their side effects. Imidacloprid (neonicotinoids) was used also against chafer grubs in 2002 (Larsen et al., 2004). Indoxacarb (oxadiazines) as a systemic insecticide was recommended and used against golf courses (Kemezys et al., 2022).

Due to the extensive use of traditional pesticide formulations, many insect pests have acquired resistance to most pesticides used. So, scientists tend to use a new strategy to overcome the side effects of traditional pesticide formulations by using nano-pesticides formulations. With this strategy, the concentration of pesticides used in pest control can be decreased and the efficacy against target pests increased.

Imidacloprid is a member of the neonicotinoids pesticide group that acts by affecting on the acetyl nicotinic receptors in the target pest. This insecticide is used against many target insect pests because it has a systemic ability and is used against soil pests (Nauen et al. 1998). Imidacloprid has a relatively long half-life in soil and acropetally movement through the xylem allows turfgrass managers to control white grubs preventively, rather than waiting until the root-feeding larvae — or possibly even turfgrass damage — are apparent. Rogers and Potter (2003) used imidacloprid to protect golf courses from the damage of third-instar Japanese beetle, *Popillia japonica*.

Chlorfenapyr is a potent insecticide used against a wide range of pests as a disruptor to the oxidative phosphorylation process and inhibits the (Adinosin triphosphate enzyme) ATP synthesis in the respiration process in the target pest (Hollingworth and Gadelhak 1998). Peterson et al. (2013) used chlorfenapyr as a soil treatment against termites. The results found that chlorfenapyr was very effective as a soil treatment against termites.

This work aims to evaluate the efficiency of nano-capsule formulations of both imidacloprid and chlorfenapyr against *S. venatus* and *P. crinita* larvae to reduce pesticide concentrations and increase efficacy.

MATERIALS AND METHODS

Tested insects

1. Hunting billbug, Sphenophorus venatus. The third and fifth instar larvae were collected by hand from the mud, roots, and stem of the grass from the golf course at Katamyia Heights Resorts (Fig. 1) and reared under laboratory conditions (26 ± 1 °C and 70 ± 5% RH) and fed on clean and fresh grass.
2. White grub, Phyllophaga crinita. The first and second instar larvae were collected from the golf course at Katamyia Heights Resorts (Fig. 1), reared under laboratory conditions, and fed on clean and fresh grass.

Tested insecticides

1. Chlorfenapyr (Challenger®36% SC) belongs to the chemical family “pyrroles” and it is the first pyrrole submitted for US registration. This pesticide was obtained from Huaian Glory Chemical Co. Ltd. China. One-fifth of the recommended field rate of nanoformulation and two lower concentrations were used (36, 18 and 9 ppm).
2. Imidacloprid (Commando® 35% SC) produced by Vapco Company Jordan. This insecticide is related to the neonicotinoids group. The recommended field rate is 250 ml/feddan (4200 m2). One-fifth of the recommended field rate of nanoformulation and two lower concentrations were used (44, 22, and 11 ppm)

Nanocapsule preparation

Nanocapsule formulations were prepared according to Chauhan et al. (2017). Chitosan with a high molecular weight was dissolved in acetic acid 1%. Sodium hydroxide (1N) was added to the chitosan solution to increase the PH from 4.6 to 4.8. Tween 80 (5%) was added to the solution as an anti-aggregation agent to prevent particle aggregation. The previous solution was filtered through Whatman filter paper (125 mm diameter). In another baker 0.8 g of sodium tripolyphosphate (TPP) was mixed with 100 ml of conducted water and 0.2 ml of imidacloprid was added. This mixture (TPP, water and imidacloprid) was added dropwise to the filtered solution (chitosan, NaOH and tween 80) under continuous magnetic stirring. The same work was carried out with chlorfenapyr. Chitosan TPP (CS-TPP) nanocapsule formation started spontaneously via the TPP-initiated ionic gelation mechanism. Nanocapsules were centrifuged at 4000 for 45 min. The supernatant was discarded, and the imidacloprid or chlorfenapyr containing chitosan nanocapsules were then freeze-dried before further analysis. The nanocapsules of both imidacloprid and chlorfenapyr were photographed by a scan electronic microscope (SEM) (Fig. 2). The size of nanocapsules ranged between 224 and 345 nm.

Determination of loading capacity of nanocapsules

The encapsulation efficacy means the mass percentage of the loaded insecticide to the total insecticide used in the preparation process (Shen et al., 2018). Nanocapsules were indirectly quantified using the supernatant after centrifugation of the final nanoemulsion solution at 4,000 rpm for 45 min by UV–visible spectrophotometer (Model: se6100 UV-Vis double beam, Abbota corporation, USA). According to Zhang et al., 2015 the efficacy encapsulation was determined by the following formula:

$$\text{Efficacy encapsulation\%} = 1 - \frac{\text{Concentration of free insecticide}}{\text{Concentration of total insecticide}} \times 100$$

The concentration of free insecticide means the concentration of insecticide in supernatant solution after centrifugation. While the concentration of total insecticide means the concentration of insecticide before centrifugation. The loading capacities were 63.4± 5.8 and 58.4 ± 3.9% with chlorfenapyr and imidacloprid nanocapsules, respectively.

Bioassay

Three concentrations for each tested nanocapsule were used against the target insects. The tested larvae were starved before treatment.

Efficacy of imidacloprid and chlorfenapyr nanocapsule against S. venatus larvae

Both the sixth and third instar larvae were used in this evaluation. The stock solution of imidacloprid nanocapsule (44 ppm) was prepared and the other two followed concentrations (22 and 11 ppm). The stock solution of chlorfenapyr was 36 ppm and the other two concentrations (18 and 9 ppm). The stock solution was one–fifth of the recommended field rate. Each concentration has three replicates. Each replicate includes 10 healthy larvae. Clean and sterile plastic boxes were used in larvae application. Each plastic box includes 50 g of soil. The tested larvae (10 larvae) were put in the soil. The soil was treated with the selected concentrations and the larvae were exposed to these concentrations and

incubated under laboratory conditions. All plastic boxes were inspected after 24 h, 48 h, 3 days, 7 days, and 10 days. The percentages of mortality were recorded and LC_{50} was determined.

Efficacy of imidacloprid and chlorfenapyr nanocapsule against P. crinita larvae

The same steps were carried out against the P. crinita larvae but with the P. crinita larvae, the first and third instar larvae were used in this evaluation.

Statistical analysis

Data were subjected to the analysis of variance test (ANOVA) (F test) and analysis of variance (one-way classification ANOVA) followed by a least significant difference (LSD) at 5% (Costat Software Program (1990)

RESULTS AND DISCUSSION

Using chlorfenapyr and imidacloprid nanocapsule against Sphenophorus venatus larvae

Chlorfenapyr and imidacloprid nanocapsules were used against the sixth and third instar larvae of S. venatus (Table 1, Fig. 3 and Fig. 4). The larvae used in control (treated with water) were not affected in all treatments.

Efficacy of chlorfenapyr nanocapsule against the third instar larvae of S. venatus

Results in Table (1) and Fig. (3) show that the third instar larvae of S. venatus were affected by chlorfenapyr nanocapsule. The percentages of mortality were 88.3, 78.3 and 61.7% for the first, second, and third concentrations, respectively. The LC_{50} was 9.1 ppm.

Efficacy of chlorfenapyr nanocapsule against the sixth instar larvae of S. venatus

As mentioned in Table (1) and Fig. (4) the percentages of mortality to the sixth instar larvae of S. venatus which caused by chlorfenapyr nanocapsule were 85, 65 and 40% for the first, second, and third concentrations, respectively. The LC_{50} of chlorfenapyr nanocapsule for the sixth instar larvae of S. venatus was 11.8 ppm. The statistical analysis shows that there is no significant difference between the efficacy of chlorfenapyr nanocapsule against the sixth and third instar larvae of S. venatus with the first concentration. While there are significant differences between the second and third concentrations.

The results showed that the third instar larvae of S. venatus were more affected by chlorfenapyr nanocapsule than the sixth instar. The LC50s were 9.1 and 11.8 ppm for the third-instar and sixth-instar larvae, respectively. This means that the third instar larvae were more susceptible to chlorfenapyr nanocapsule than the sixth instar larvae (Fig. 5).

Efficacy of imidacloprid nanocapsule against the third instar larvae of S. venatus

The percentages of mortality were increased sharply with all tested concentrations of imidacloprid. The percentages of mortality were 96.7, 80 and 60% with the first, second and third concentrations, respectively. So, the LC_{50} was 8.8 ppm (Table 1 and Fig 6).

Efficacy of imidacloprid nanocapsule against the sixth instar larvae of S. venatus

The percentages of mortality were 95, 81.7 and 53.3% for the first, second and third concentrations, respectively. The LC_{50} was 8.8 ppm (Table 1). The obtained results showed that there is no difference between the efficacy of imidacloprid against the third and sixth instar larvae of S. venatus especially with the first and second concentrations (the highest concentrations). While there is a little difference with the third concentration (Fig. 7).

Data showed that the third instar larvae of S. venatus were affected by imidacloprid with the third concentration compared with the sixth instar larvae (60 and 53.3%, respectively) (Fig. 8). The less significant difference (LSD) was 7.4 with the third concentration compared with 3.3 and 3.3 with the first and second concentrations (Table 1). The LC50,s were 8.8 ppm with the third instar larvae compared with 10.3 ppm with the sixth instar larvae.

To throw some light on the efficacy of both chlorfenapyr and imidacloprid nanocapsule against the third and sixth instar larvae of S. venatus; data showed that imidacloprid was more effective than chlorfenapyr with both the third and sixth instar larvae of S. venatus. For the first concentration the percentage of mortalities caused by imidacloprid were 96.7 and 95% against the third and sixth instar larvae, respectively, compared with 88.3 and 85% in chlorfenapyr treatment. The same results were found with the second and third concentrations (Table 1).

These results were consistent with Heller et al. (2008). The authors found that the percentage of mortalities caused by imidacloprid against S. venatus larvae ranged between 62.1% and 79.4%. Reynolds and Brandenbur (2015) found that the percentage of mortality caused by imidacloprid against S. venatus larvae wasn't exceeded 33.6% with a concentration of 0.77 lb ai/ha. In this work, one–tenth of this concentration caroused 96.6 %. This means that imidacloprid nanoformulation was more effective than the normal formulation. Sabry et al. (2021) found that nanoparticles of imidacloprid were very effective against Spodoptera littoralis larvae compared with the normal formulation. Sabry and Hussein (2021) found also the nanoformulation of both imidacloprid and chlorfenapyr was more effective against Monacha cartusiana than the normal formulations. Memarizadeh et al. 2014. Used imidacloprid nanocapsule against Glyphodes pyloalis. The results showed that the nanocapsule of imidacloprid was more effective against this pest and also safer in the environment than the normal formulation.

Using of chlorfenapyr and imidacloprid nanocapsule against Phyllophaga crinita larvae

Table (2) shows that the efficacy of both chlorfenapyr and imidacloprid against the first and third instar larvae of P. crinita

Efficacy of chlorfenapyr nanocapsule against the first instar larvae of P. crinita

Three concentrations of chlorfenapyr nanocapsule were used. The first concentration of chlorfenapyr caused the highest mortality percentage followed by the second and the third concentrations. The percent of mortalities were 86.7, 65 and 41.7% for the first, second and third concentrations, respectively (Table 2 and Fig. 9). The LC_{50} was 11.6 ppm.

Efficacy of chlorfenapyr nanocapsule against the third instar larvae of P. crinita

The obtained data in Table (2) and Fig. (10) show that the percent of mortality caused by chlorfenapyr nanocapsule was 75, 48.3, and 31.7% for the first, second, and third concentrations, respectively. The LC_{50} was 17.2.

The obtained data showed that the third instar larvae of P. crinita were more tolerant to chlorfenapyr nanocapsule than the first instar. The LC_{50} were 17.2 and 11.6 for the third and first instar larvae, respectively (Table 2 and Fig. 9). The statistical analysis showed that there were significant differences among all tested concentrations of chlorfenapyr nanocapsule against the third and first instar larvae. The less significant difference (LSD 5%) values were 6.7, 6.7, and 4.7 for the first, second and third concentrations, respectively (Fig. 11).

Efficacy of imidacloprid nanocapsule against the first instar larvae of P. crinita

As clear in Table (2) and Fig. (12) imidacloprid was very effective against the first instar larvae of P. crinita. The percentages of mortality were 95, 80 and 60% for the first, second, and third concentrations, respectively. The LC_{50} was 8.7 ppm.

Efficacy of imidacloprid nanocapsule against the third instar larvae of P. crinita

The third instar larvae of P. crinita was affected by imidacloprid. The percent of mortality with the first concentration (the highest concentration) was 91.7% (Table 2 and Fig. 13). The percentages of mortality for the second and third concentrations were 71.7 and 51.7, respectively. The LC_{50} was 11.2 ppm. The obtained data found that the first instar larvae of P. crinita were more affected than the third instar. This may be due to the size of the larvae. The third instar was bigger than the first instar. So, the third instar was more tolerant to imidacloprid than the first instar (Fig. 14). The LC_{50},s were 8.7 and 11.2 ppm for the first and third instar larvae, respectively.

The statistical analysis showed that there was a significant difference between the efficacy of chlorfenapyr nanocapsule against the first and third instar larvae of P. crinita. While there was no significant difference with the imidacloprid

These results were consistent with Niemczyk and Shetlar (2000). The author used imidacloprid against the earlier-hatching black turf-grass ataenius, Ataenius spretulus (which infests the golf courses). They found that imidacloprid is very effective against this pest because it has a long residual effect. Koppenhofer et al. (2008) used imidacloprid against the second and third-instar larvae of P. crinita in cranberry fields.

CONCLUSION

This paper may be the first paper on using nanocapsule insecticides against golf courses pest. The obtained results found that imidacloprid nanocapsule was more effective against both the *Sphenophorus venatus* and *Phyllophaga crinita* larvae. The LC_{50} of imidacloprid against the sixth and third instar larva of *S. venatus* was 10.3 and 8.8 ppm, respectively, compared with 11.8 and 9.1 ppm with chlorfenapyr. The same

results were found with the first and third instar larvae of *P. crinita*. The LC_{50} of imidacloprid was 8.7 and 11.2 compared with 11.6 and 17.2 ppm with chlorfenapyr. Data also showed that the young larvae were more susceptible than the full-grown in both tested pests. The aim of using nanocapsule insecticides is to reduce the concentration of pesticides used, reduce soil contamination by traditional pesticide formulation, the cost of application, and increase the efficacy of pesticides against golf course pests.

Declarations

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. All authors declared that there is no conflicts of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

AUTHOR CONTRIBUTIONS

All authors designed the research, conducted the experiments, analyzed the data, wrote, revised, and approved the manuscript.

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Tables

Table 1. Efficacy of chlorfenapyr and imidacloprid nanocapsule against the larvae of *Sphenophorus venatus*

Treatments	Percentages of mortality									
	Chlorfenapyr					Imidacloprid				
	C1	C2	C3	Slope	LC_{50} and fiducial limits	C1	C2	C3	Slope	LC_{50} and fiducial limits
Sixth instar larvae	85±5^{a}	65±5^{b}	40±5^{b}	2.1±0.3	11.8 (9.3 – 14.1)	95±0.0^{a}	81.7±2.9^{a}	53.3±5.8^{b}	2.6±0.4	10.3 (7.7 – 12.3)
Third instar larvae	88.3±2.9^{a}	78.3±2.9^{a}	61.7±2.9^{a}	2.1±0.3	9.1 (6.4 – 11.2)	96.7±2.9^{a}	80± 0.0^{a}	60±0.0^{a}	2.5±0.4	8.8 (6.1 – 11.0)
Control	0.0^{b}	0.0^{c}	0.0^{c}			0.0^{b}	0.0^{b}	0.0^{c}		
F-values	676.3***	474.3***	624.3***			3307.0***	3353.0***	241.8***		
LSD	6.7	6.7	6.7			3.3	3.3	7.4		

*Means under each treatment sharing the same letter in a column are not significantly different at P = 0.05

Table 2. Efficacy of chlorfenapyr and imidacloprid nanocapsule against the larvae of *Phyllophaga crinita*

Treatments	Percentages of mortality									
	Chlorfenapyr					Imidacloprid				
	C1	C2	C3	Slope	LC50 and fiducial limits	C1	C2	C3	Slope	LC50 - and fiducial limits
Third instar larvae	75±5[b]	48.3±2.9[b]	31.7±2.9[b]	1.9±0.3	17.2 (14.2 – 20.6)	91.7±2.9[a]	71.7±2.9[b]	51.7±2.9[b]	2.2±0.3	11.2 (8.2 – 13.6)
First instar larvae	86.7±2.9[a]	65±5[a]	41.7±2.9[a]	2.3±0.3	11.6 (9.2 – 13.7)	95± 50[a]	80± 0.0[a]	60±5.0[a]	2.2±0.4	8.7 (5.7 – 11.0)
Control	0.0[c]	0.0[c]	0.0[c]			0.0[b]	0.0[c]	0.0[c]		
F-values	597.25***	307.75***	255.5***			784.75***	2089.0***	285.0***		
LSD	6.7	6.7	4.7			6.7	3.3	6.7		

*Means under each treatment sharing the same letter in a column are not significantly different at P = 0.05

Figures

Figure 1

Hunting billbug and white grub larvae infested the golf courses

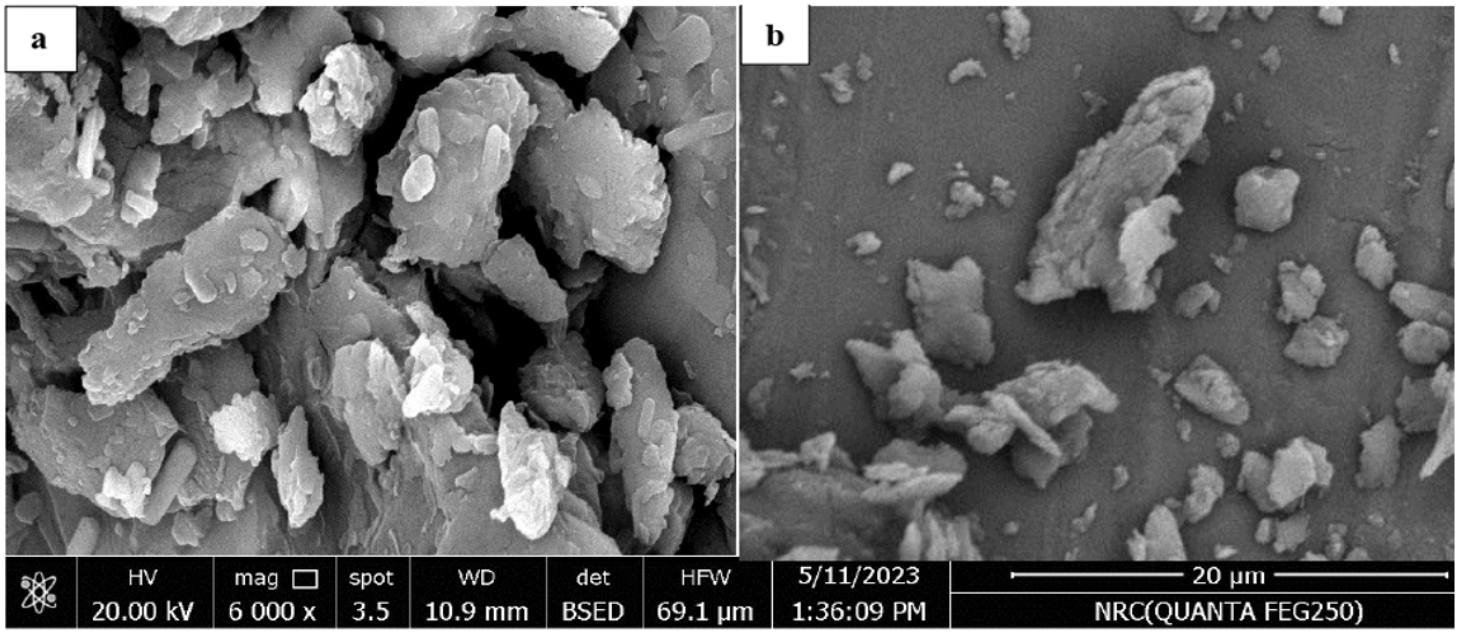

Figure 2

chlorfenapyr (a) and imidacloprid (b) nanocapsules under SEM

Figure 3

Effect of chlorfenapyr against the third instar larvae of *S. venatus* (control (a), treated after one day (b) and after five days of treatment)

Figure 4

Effect of chlorfenapyr against the sixth instar larvae of *S. venatus* (control (a), treated after one day (b) and after five days of treatment)

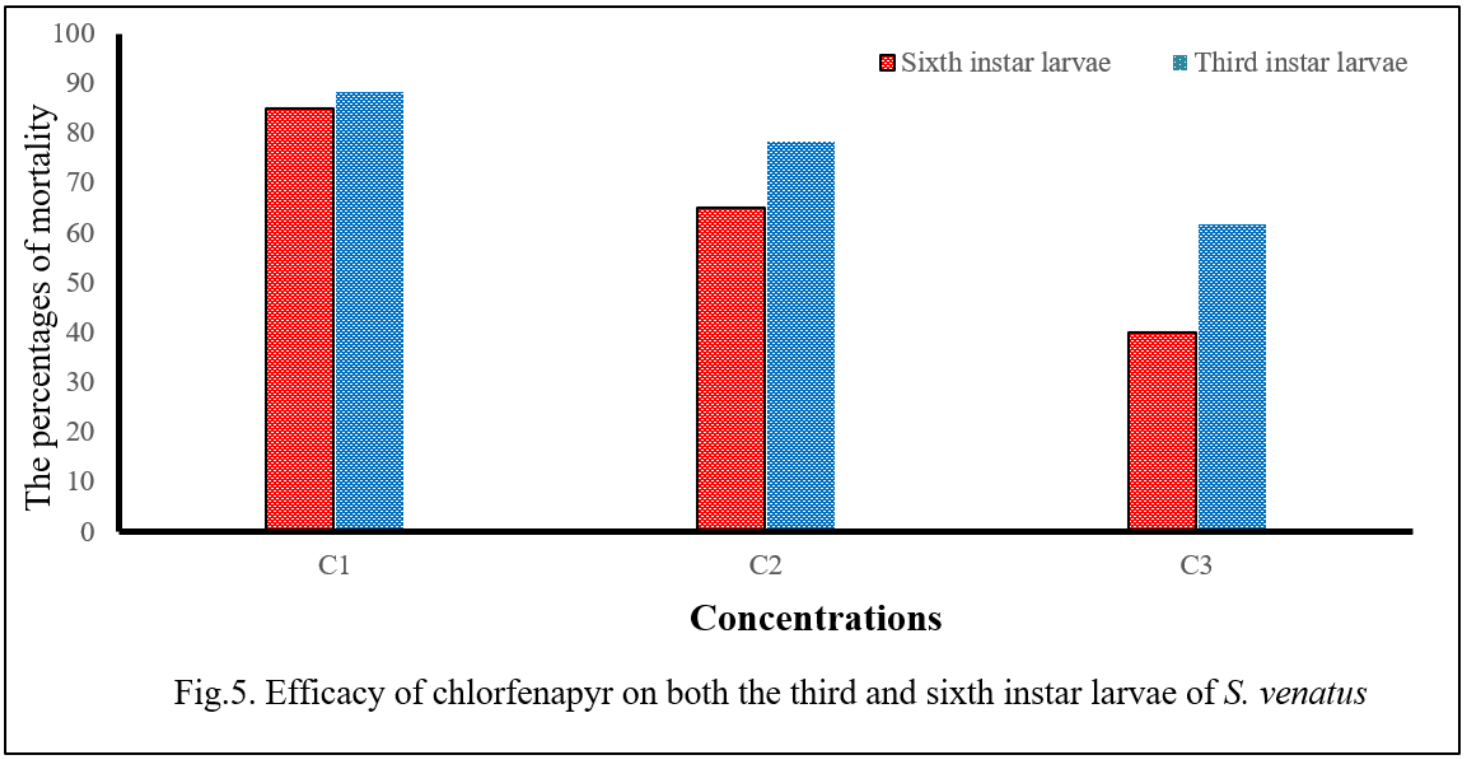

Fig.5. Efficacy of chlorfenapyr on both the third and sixth instar larvae of *S. venatus*

Figure 5

See image above for figure legend.

Figure 6

Effect of imidacloprid against the third instar larvae of *S. venatus* (control (a), treated after one day (b) and after five days of treatment)

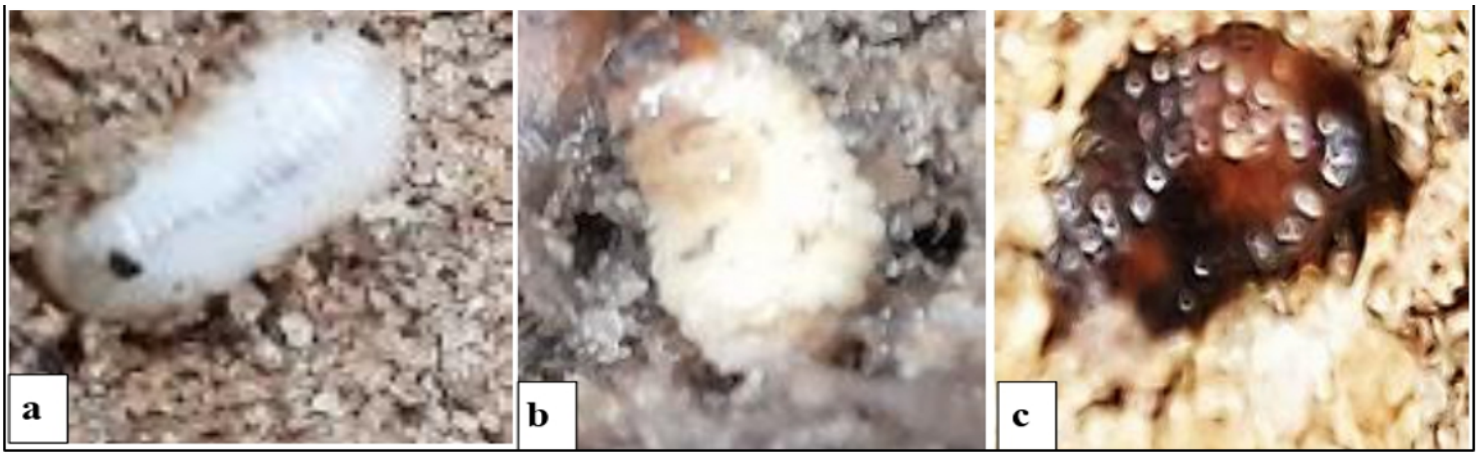

Figure 7

Effect of imidacloprid against the sixth instar larvae of *S. venatus* (control (a), treated after one day (b) and after five days of treatment)

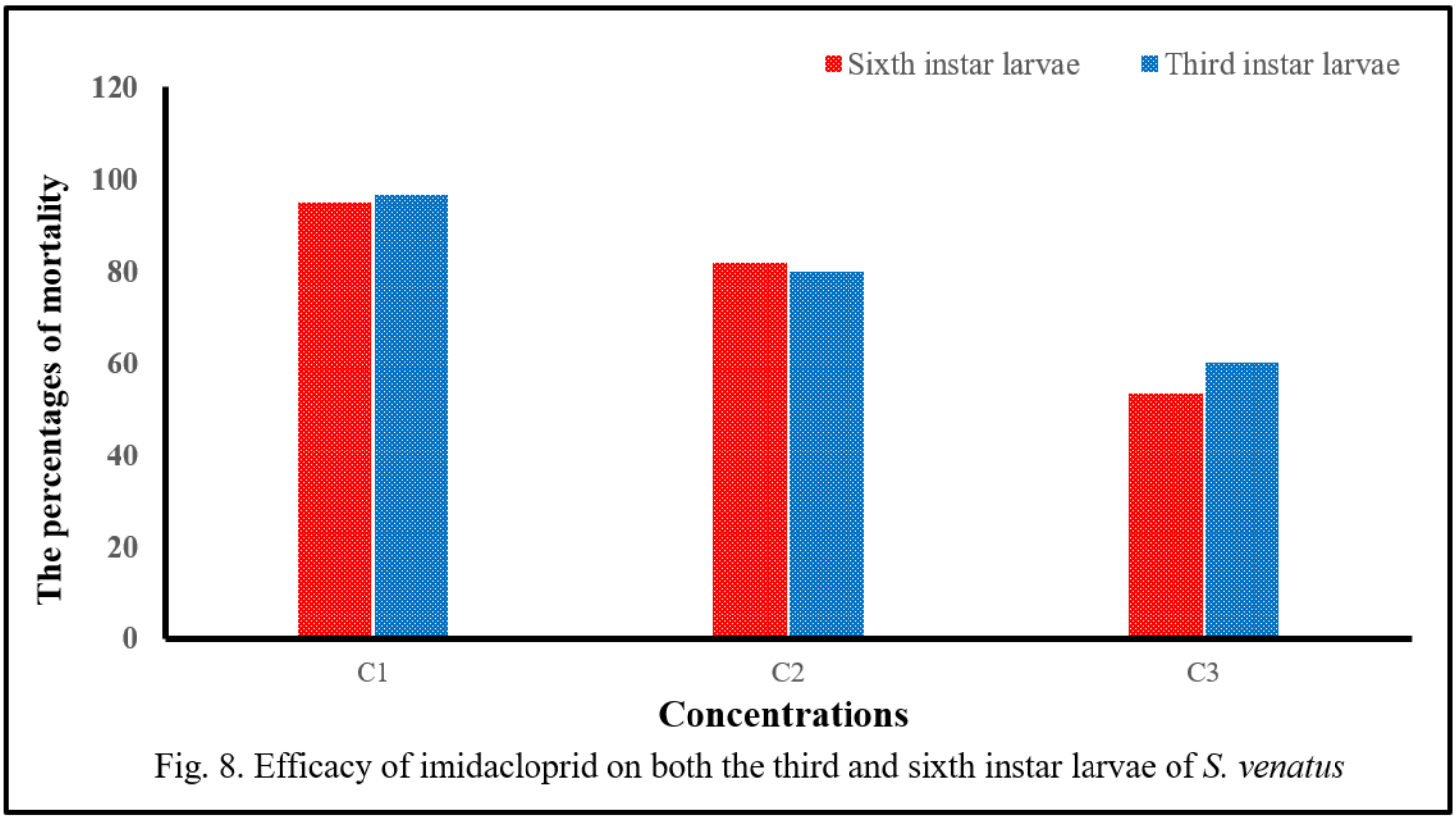

Fig. 8. Efficacy of imidacloprid on both the third and sixth instar larvae of *S. venatus*

Figure 8

See image above for figure legend.

Figure 9

Effect of chlorfenapyr against the first instar larvae of *P. crinita* (control (a), treated after one day (b) and after five days of treatment)

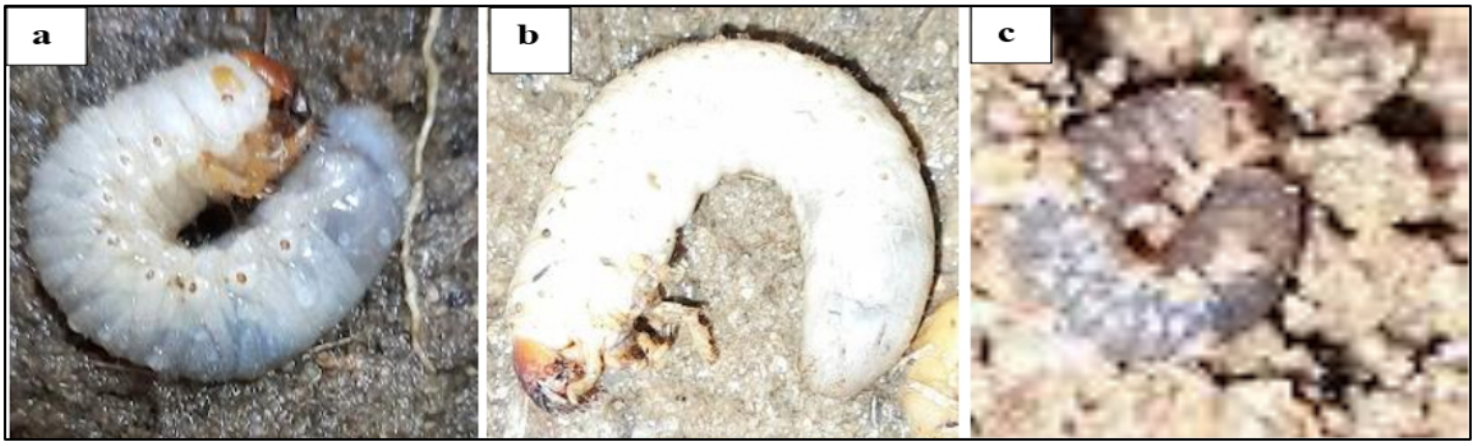

Figure 10

Effect of chlorfenapyr against the third instar larvae of *P. crinita* (control (a), treated after one day (b) and after five days of treatment)

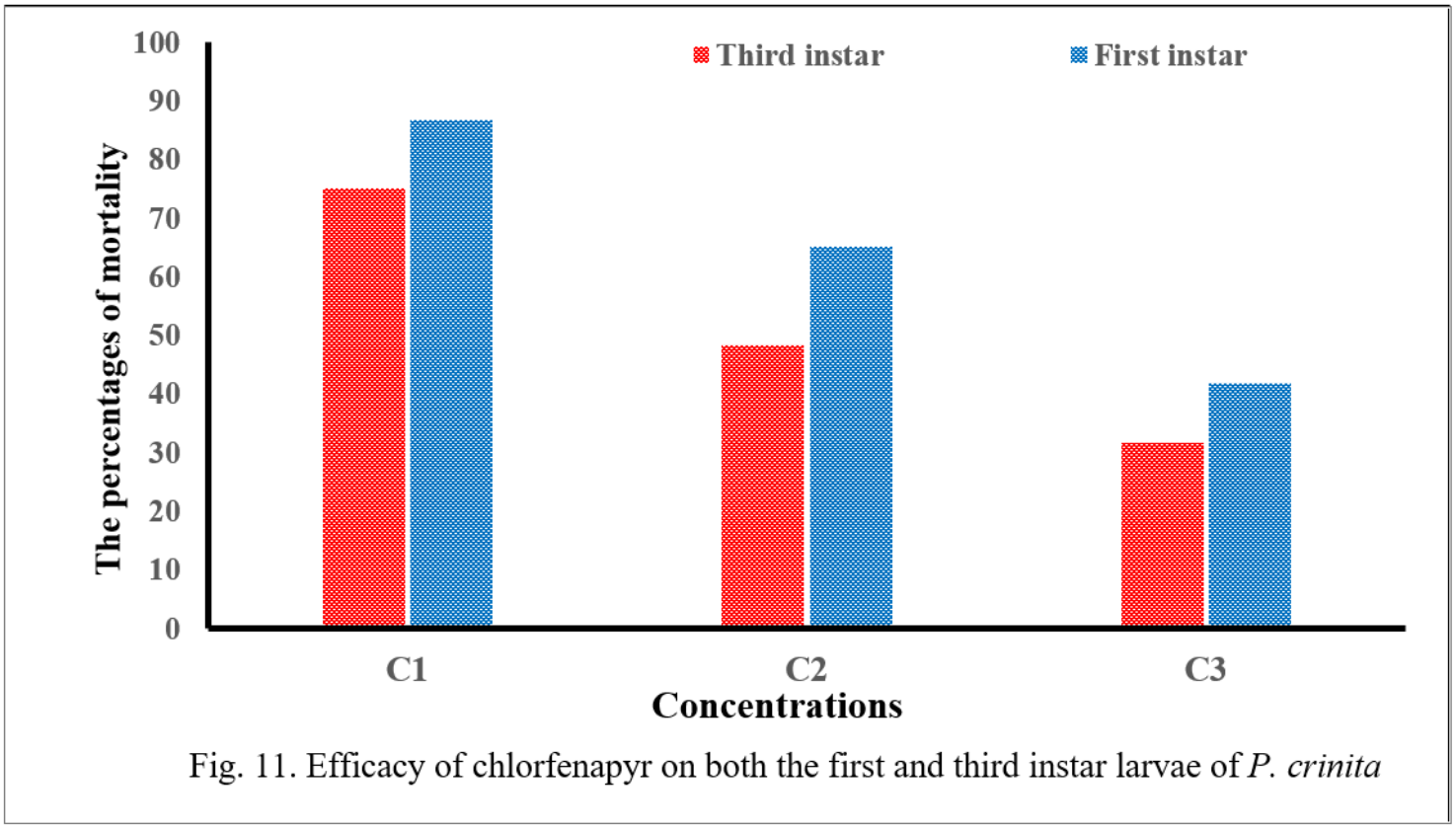

Figure 11

See image above for figure legend.

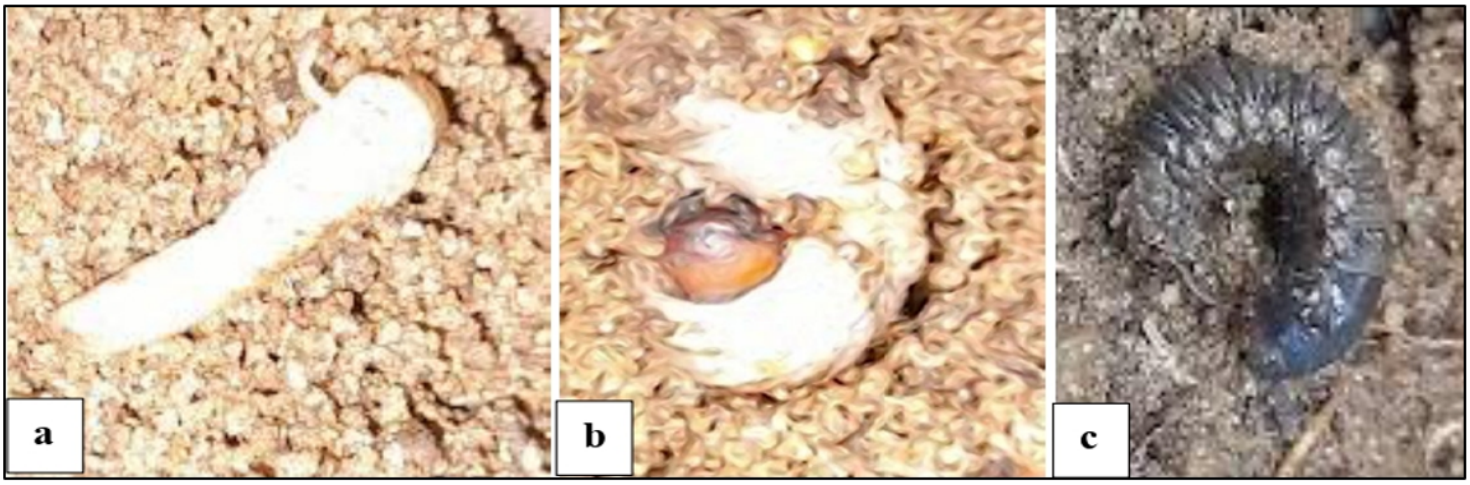

Figure 12

Effect of imidacloprid against the first instar larvae of *P. crinita* (control (a), treated after one day (b) and after five days of treatment)

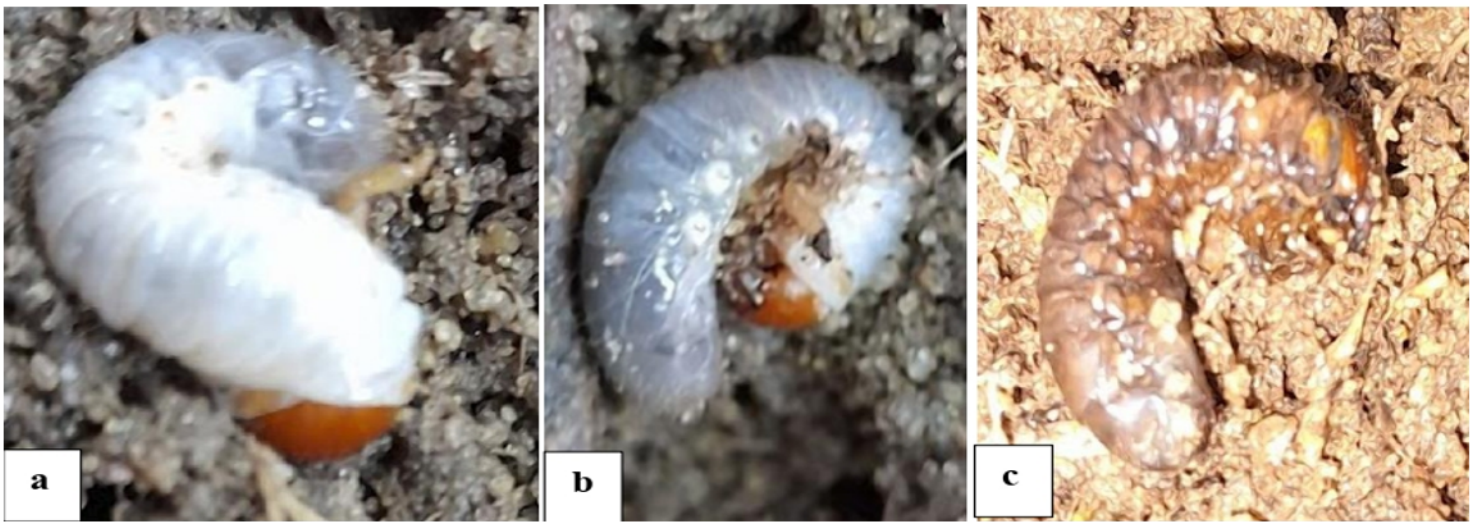

Figure 13

Effect of imidacloprid against the third instar larvae of *P. crinita* (control (a), treated after one day (b) and after five days of treatment)

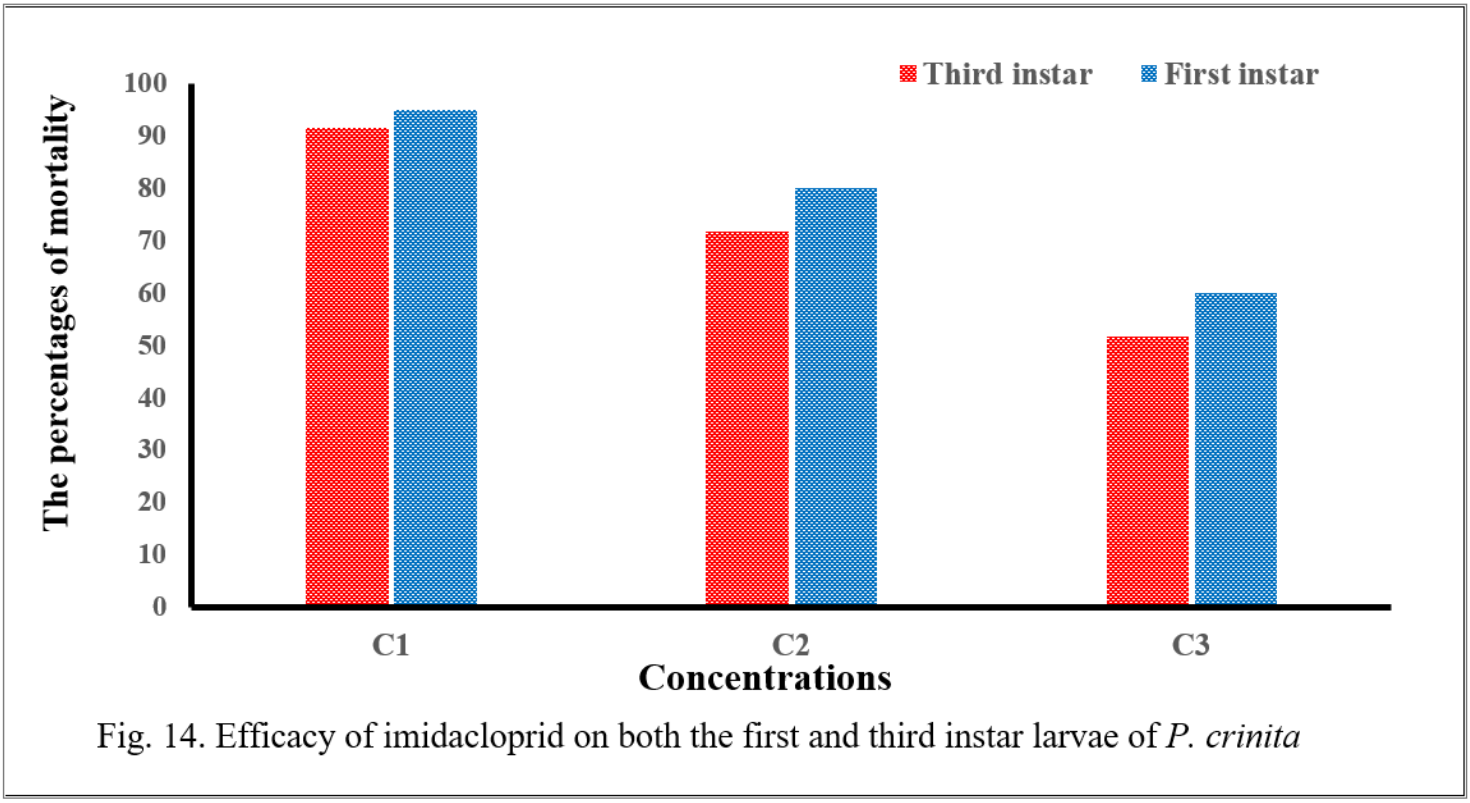

Fig. 14. Efficacy of imidacloprid on both the first and third instar larvae of *P. crinita*

Figure 14

See image above for figure legend.