



Effects of abamectin and fipronil insecticides on the brain and compound eyes of the embryo of *Heteracris littoralis* (Rambur) (Orthoptera: Acrididae)

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Received: 3 October 2022 / Accepted: 13 March 2023 / Published online: 16 June 2023
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Abstract

Chemical pesticides are essentially hazardous. The risks differ from compound to compound, and much of the information on their actions on insect development remains to be captured. The toxicity of abamectin (a macrocyclic lactone, acting on glutamate-gated chloride channels of insects), and fipronil (a phenylpyrazole, disrupting the GABA receptors) was given on embryos of the short-horned grasshopper *Heteracris littoralis* (Rambur, 1838) for the first time. Abamectin is 66 times more fatal than fipronil. Treated eggs with a sublethal dose gave a reduction up to 51% in hatchability as compared with normal eggs; yet, without any effect on the incubation period. Many embryos have stopped developing at certain developmental stages. The disruptive effects of both the tested chemicals on the brain and the compound eyes were described. The brain and the compound eyes were severely disrupted. The optic lobes appear small in size, and this led to the malformed compound eyes and optic nerves. The brain cells appeared loose and perhaps few in number. The neurosecretory materials carried in the neurosecretory cells were not clear. The neuropil was wide in the normal brain rather than in treated ones. Treated embryos suffered a shrinkage in ommatidia size and number, they are also irregular. Perhaps continued and precise studies should be made to minimize resistance, usually low doses enhance. Continuing studies on the tested pesticides may provide novel insights on their actions for more effective insect control strategies.

Keywords Toxicity · Disrupted development · Locusts

Introduction

Abamectin and fipronil are insecticides used in Egypt to control both household and agricultural pests. Abamectin is a natural product of soil actinomycete (Ikeda et al. 2003). It acts as a blocker of electrical activity transmission in nerves and muscles (Wolstenholme and Rogers 2006). Fipronil is a phenylpyrazole insecticide that kills insects through binding with γ -aminobutyric acid receptors and glutamate-gated chloride channels (Raymond-Delpech et al. 2005).

Knowledge on embryonic development of grasshoppers and locusts under stress of different chemicals and/or stressors is fragmentary (Bowers and Ortego 1991; Raina et al. 1995; Vennard et al. 1998; Ghazawy et al. 2010; Abdel

Rahman 2017; Bode 2020). Perhaps these insects deposit their egg pods into soil; hence, they are protected. The intensive use of chemical pesticides led to the accumulation of many residues especially many stable chemicals with a long half-life to dissociate. Such chemicals might go into soil and perhaps affect life beneath the surface (Badawy 1998). One of these lives are the developing embryos of grasshoppers which might last from 2 to 4 weeks according to the species and soil temperature (Abdel Rahman 2000). This period is suitable to study the effect of any chemicals used even at low concentrations (LC_{30}) and they are still active after use. Hatchability and incubation periods of embryos might be affected by such chemicals and perhaps we can use these effects as measures for contamination or for prediction of the grasshopper population status (Li et al. 2021).

The tested pesticides were used on many pests (de Souza and Guimarães 2022; Simon-Delso et al. 2015), yet the effect on embryos was not tested yet. The continuous use of pesticides might lead to many residual effects in soil. Grasshoppers oviposit in the soil so their egg pods might

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Table 1 Toxicity of abamectin and fipronil on *H. littoralis* eggs

Insecticide	LC ₅₀ (95% FL)	Fit of the probit line			LC ₃₀
		Slope ± SE	χ ²	df	
Abamectin (ppm)	30.97 (20.40–44.70)	0.85 ± 0.13	5.54	16	7.485
Fipronil (x10 ⁻²)%	4.67 (3.18–6.34)	1.05 ± 0.14	2.12	16	1.482

suffer from insecticides used where they usually dwell. They might be affected together with hatchability as well. Hence, I hypothesized that doses of nerve poison pesticides would affect the microstructure of sense organs of the grasshopper *H. littoralis* embryos. In this paper I describe outcomes of experiments designed to test this hypothesis.

Martials and methods

The short-horned grasshopper *Heteracris littoralis* (Rambur, 1838) was reared in laboratories of the Department of Entomology, Faculty Science, Cairo University for several years in room temperature and the ambient relative humidity. The grasshoppers were fed clover (*Trifolium alexandrinum*) from October till June, then sesban (*Sesbania sesban*). To get fresh eggs, they were offered pots contained clean, sieved, sterilized and wet sand to oviposit. Egg-pods were removed from these pots in the morning to a Petri dish with a wet cotton and were concerned as 0-day. They were incubated at 27 °C. For experimental purposes, 20 eggs from different egg-pods (5 eggs/pod) were used for each concentration of the pesticides to determine the median lethal concentration (LC₅₀). Eggs were put in a watch glass and one ml of the tested insecticide (different concentrations) was added on these eggs for 2–3 s then they were moved in a Petri dish with a wet cotton and was incubated as above. For comparison, the author used the percent development (%D) for a given stage of development; this was calculated by dividing the mean incubation period of a given stage over the total incubation period of the eggs and multiplied by 100 (Abdel Rahman 2017). The pesticides used were abamectin (1.8%) and fipronil (20%) and were diluted with water. All experiments were three times replicated. Control groups were treated with water only. The lethal dose (LC₃₀) was calculated

Table 2 Effects of abamectin (Ab) and fipronil (Fb) on *H. littoralis* embryos

	Number hatched	Incubation period (days)	Numbers* of embryos stopped development at a given stage (%D).			
			23	36	59	90
Control	15.0 ± 1.73	25.42 ± 0.01	-	-	-	-
Ab	7.7 ± 1.52	25.48 ± 0.16	3	2	3	2
Fb	6.7 ± 0.57	24.85 ± 0.05	3	3	3	2

*The total number of embryos of the 3 replicates; i.e., out of 60. N=20

and was used in histological investigations. For this purpose, 20 eggs were used and were three times replicated.

Histological preparation were made by fixing eggs in hot Bouin's solution for 24 h, then they were washed several times in 70% alcohol, then dehydrated in ascending alcoholic series up to 100%, cleared in cedar wood oil (for 24 h) and were infiltrated in paraffin wax (for 3 h) (melting point 60°). They were mounted, trimmed and were cut 8–10 μm using a microtome and were mounted on a glass slide. Specimens were stained with hematoxylin and eosin and were differentiated in acid-alcohol (1–2 drops of conc. HCl in 100 ml 70% alcohol). Finally, a drop of Canada balsam was added on the stained specimens and covered with a coverslip. Slides were examined and then photographed with Galaxy M31 Mobile.

Statistical analysis and the probit analysis were carried out with IBM SPSS Statistics Version 22 (IBM Corp. Armonk, NY, USA).

Results

The toxicity of the two insecticides abamectin and fipronil is given in Table 1. It is clear that abamectin is about 66 times more fatal than fipronil, as the LC₅₀ was 30.973 ppm (≈0.003973%) and 0.04668% for both insecticides, respectively.

Abamectin and fipronil at LC₃₀ level have stronger effects on hatchability of the treated eggs that were reduced to about 51 and 44% as compared with the normal eggs; respectively (Table 2). There were no significant effects on the total incubation periods (P < 0.05).

As many eggs didn't hatch while they contained embryos, these embryos stopped developing at certain developmental stages: at 23 and 36%D before the katatreptic movement and 59 and 90%D after that. Embryos reached 90%D were a few and represented about 6.7% of the total number of embryos examined (Table 2).

Based on the histological preparations for both treated and control embryos, the most disrupted organs were the brain and the compound eyes (Fig. 1C). The brain develops from the ectoderm of the first three segments (the optic, the antennary and the intercalary) while the compound eyes develop ectodermally from the first segment (optic segment). The treated embryos showed a shrunked and malformed brain

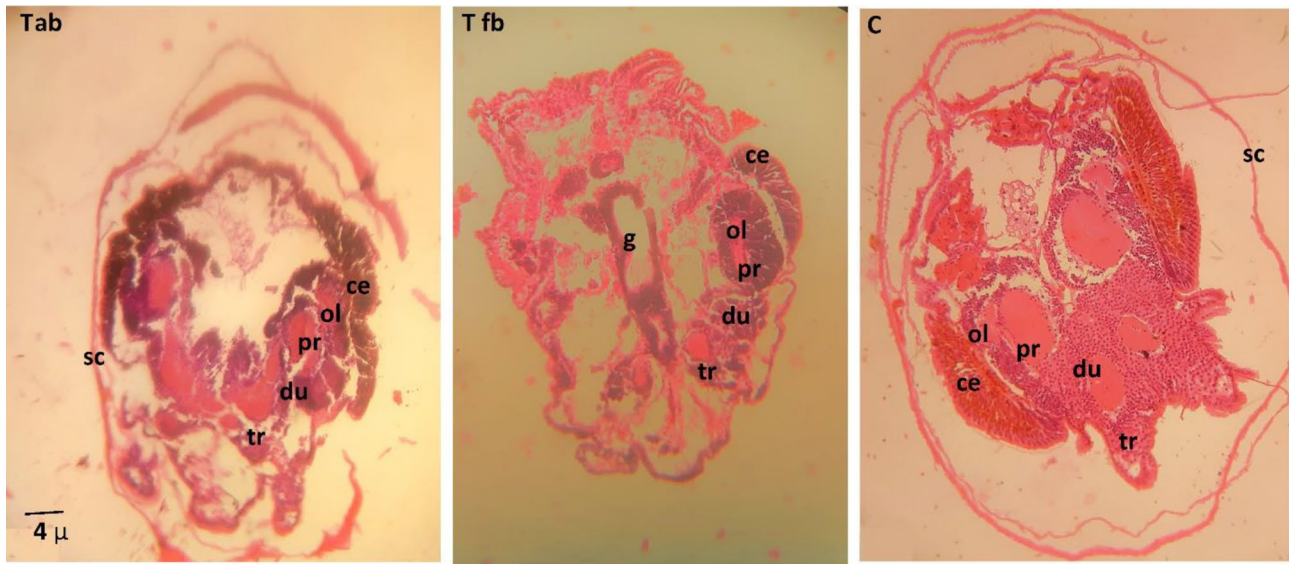


Fig. 1 Transverse section through the head of embryos at 90%D showing the effects of abamectin (**ab**) and fipronil (**fb**) on the brain and compound eyes. **C**; control; **ce**, compound eye; **du**, deutocerebrum;

g, foregut; **ol**, optic lobe; **pr**, protocerebrum; **sc**, serosal cuticle; **T**, treated; **tr**, tritocerebrum

in its basic structures (Fig. 1T): the protocerebrum and the optic lobe, the deutocerebrum and the tritocerebrum. The optic lobes appeared small in size as compared with the control embryos (Fig. 1T ab; T fb). Perhaps this lead to the malformed compound eyes and the optic nerves.

The brain cells were affected seriously by treatment. They appeared loose and perhaps few in number as shown in Fig. 2T ab, T fb. The neurosecretory materials carried in the neurosecretory cells were not clearly seen as compared with normal cells (Fig. 2C). The neuropil was wide in the normal brain rather than in treated ones (Fig. 2C).

A compound eye consists of grouped ommatidia that are connected to the optic lobe via an optic nerve (Fig. 3C).

Basically, an ommatidium consists of a corneal lens that is a transparent cuticular layer, crystalline cone and retinula cell. These ommatidia rest on a basement membrane and they are connected to the optic lobe through the optic nerve. Treated embryos suffer shrinkage in ommatidia size and number, they are also irregular in shape as seen in Fig. 3T ab, T fb.

Discussion

Abamectin is a widely used insecticide and is a natural fermentation product of soil dwellings (Ikeda et al. 2003). It binds to the glutamate-gated chloride channels found

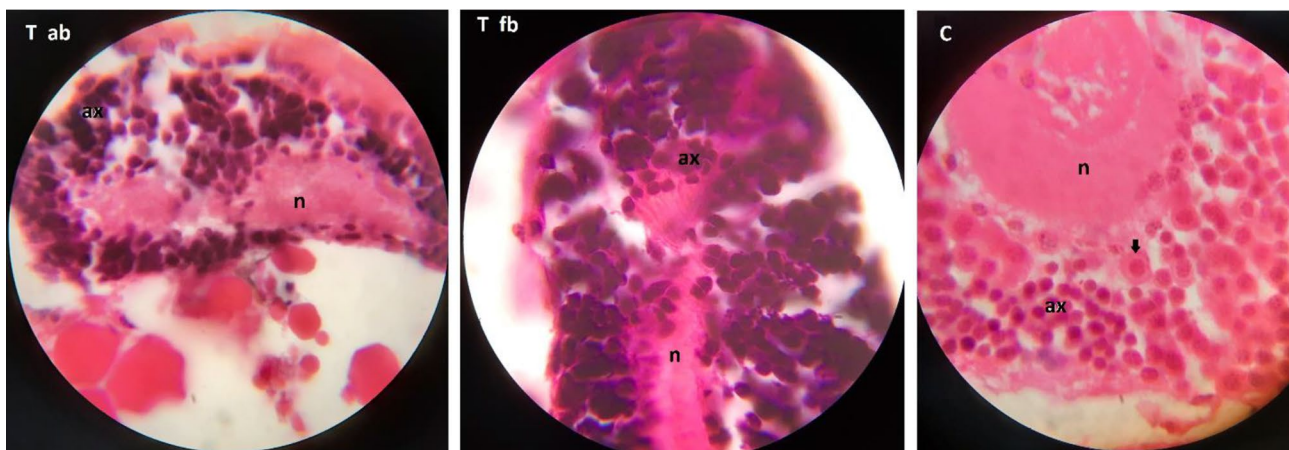


Fig. 2 A magnified portion of a transverse section through the head of *H. littoralis* embryos at 90%D showing the effects of abamectin (**ab**) and fipronil (**fb**) on the protocerebrum. **ax**, nerve cells; **n**, neuro-

pil; **C**; control; **T**, treated; arrowhead shows the neurosecretory material in normal brain cells

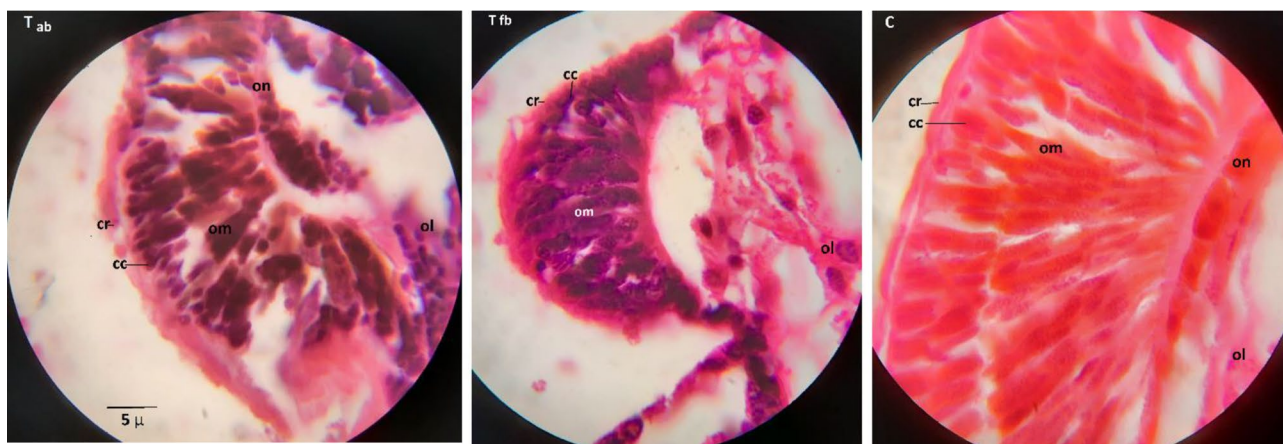


Fig. 3 A magnified portion of a transverse section through the head of *H. littoralis* embryos at 90%D showing the effects of abamectin (**ab**) and fipronil (**fb**) on the compound eyes. **cc**, corneal lens; **cr**, crystalline cone; **C**, control; **I**, lens; **om**, ommatidium; **on**, optic nerve; **T**, treated

in nerve and muscle cells (Wolstenholme and Rogers 2006). Death takes place when these cells are hyperpolarized, then paralysis and finally death (Wolstenholme and Rogers 2006). Fipronil belongs to the phenylpyrazole family and disrupts the central nervous system by blocking the ligand-gated ion channel of the γ -aminobutyric acid receptor “GABA” and glutamate-gated chloride (GluCl) channels. This causes hyperexcitation of insects’ nerves and muscles (Wolstenholme 2012).

Chemical pesticides were used continuously in Egypt a long time ago against many pests (Mansour 2008). Perhaps this selected many pests to develop resistance to these chemicals. Non-targeted insects might get a chance to be more resistant and might become harmful ones (Ishtiaque and Saleem 2011). The grasshopper *H. littoralis* is a pest in Egypt for many economic plants like clover, cotton, flax and bean (Ibrahim 1980). Embryos of this grasshopper might help to add more data about a non-targeted insect with sublethal dose.

The development of the brain in grasshopper embryos starts at 17–21%D by the appearance of the neuroblasts in most grasshoppers studied (Abdel Rahman 2000). In the head, the brain starts to differentiate by 26–33%D and is fully completed at 88–94%D. The compound eyes develop from the lateral ectoderm of the optic segment by 29–35%D and start to be red in color by 47–67%D (Abdel Rahman 2000). Other compounds as pyriproxyfen (IGR mimic) caused embryos of *Schistocerca gregaria* to stop developing at 45–73%D according to the dose and day of treatment (Vennrad et al. 1998).

During this development, the above insecticide might interfere with the formation of the brain, hence the disrupted development occurs. Many chemicals (IGRs) had the same effect e.g. *S. gregaria* (Ghazawy et al. 2010; Abdel Rahman 2017). Ions like Cl^- might invade cells (Bloomquist 1993) causing death during embryogenesis.

Conclusion

In this work, the toxicity of abamectin and fipronil towards the short-horned grasshopper *H. littoralis* is evident. Effects on hatchability and incubation period supports the negative impact of these chemicals on development. The disruptive effects on the brain and the compound eyes were clearly evident, where, the embryotoxicity of these chemicals, exaggerated by morphological alteration, interfering with development and differentiation of embryos. Perhaps we should pay more attention to further calculate precisely the required doses for controlling this pest in the field, since the low doses perfectly altered the histologic structure in embryos tested.

Acknowledgements The author appreciates the help of Dr. M. T. Elnems, head of the Egyptian Pest Management Association (EPMA) for offering the insecticides; also thanks are extended to Dr. M. Soliman, Entomol. Dept., Fac. Sci., Cairo Univ., for calculating the LC_{50} .

Funding Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB). The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data availability The data obtained is available in the manuscript.

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

Conflict of interest There is no conflict of interest.

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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References

- Abdel Rahman KM (2000) Studies on the embryology of the grasshopper *Chroptogonus lugubris* Blanchard, (Orthoptera: Acridoidea, Pyrgomorphidae). I. morphogenesis, rate of development and water requirements. *Acta Fytotech Zootech* 1:6–12
- Abdel Rahman KM (2017) Embryonic development disrupted in the desert locust *Schistocerca gregaria* Forskål (Orthoptera: Acrididae) due to lufenuron application. *Efflatounia* 17:1–8
- Badawy MI (1998) Use and impact of pesticides in Egypt. *Int J Environ Health Res* 8:223–239. <https://doi.org/10.1080/09603129873507>
- Bloomquist JR (1993) Toxicology, mode of action and target site-mediated resistance to insecticides acting on chloride channels. *Comp Biochem Physiol C Toxicol Pharmacol* 106:301–314
- Bode K, Bohn M, Reitmeier J et al (2020) A locust embryo as predictive developmental neurotoxicity testing system for pioneer axon pathway formation. *Arch Toxicol* 94:4099–4113
- Bowers WS, Ortego F (1991) Evaluation of Juvenoid Insect Growth regulators on *Schistocerca Americana*. *Int J Trop Insect Sci* 12:71–75
- de Souza RB, Guimarães JR (2022) Effects of avermectins on the environment based on its toxicity to plants and soil invertebrates—a review. *Water Air Soil Pollut* 233:259
- Ghazawy NA, Awad HH, Abdel Rahman KM (2010) Effects of azadirachtin on embryological development of the desert locust *Schistocerca gregaria* Forskål (Orthoptera: Acrididae). *J Orthop Res* 19:327–332
- Ibrahim MM (1980) Development and survival of the grasshopper *Heteracris littoralis* Rambur on a restricted diet (Orthoptera: Acrididae). *Z Ang Ent* 90:22–25
- Ishtiaque M, Saleem MA (2011) Generating susceptible strain and resistance status of field populations of *Spodoptera exigua* (Lepidoptera: Noctuidae) against some conventional and new chemistry insecticides in Pakistan. *J Econ Entomol* 104:1343–1348
- Ikedo H, Ishikawa J, Hanamoto A, Shinose M, Kikuchi H, Shiba T et al (2003) Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. *Nat Biotechnol* 21:526–531
- Li XD, Xin L, Rong RW et al (2021) Effect of heavy metals pollution on the composition and diversity of the intestinal microbial community of a pygmy grasshopper (*Eucrotettix oculatus*). *Ecotoxicol Environ Saf* 223:1–10
- Mansour SA (2008) Environmental impact of pesticides in Egypt. *Rev Environ Contam Toxicol* 196:1–51
- Raina SK, Das S, Rai MM, Khurad AM (1995) Transovarial transmission of *Nosema locustae* (Microsporida: Nosematidae) in the migratory locust *Locusta migratoria migratorioides*. *Parasitol Res* 81:38–44
- Raymond-Delpech V, Matsuda K, Sattelle BM, Rauh JJ et al (2005) Ion channels: molecular targets of neuroactive insecticides. *Invertebr Neurosci* 5:119–133
- Simon-Delso N, Amaral-Rogers V, Belzunces LP et al (2015) Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environ Sci Pollut Res* 22:5–34
- Vennard C, Nguama B, Dillon HJ, Ooughi IH, Chahnley AK (1998) Effects of the juvenile hormone mimic pyriproxyfen on egg development, embryogenesis, larval development, and metamorphosis in the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae). *J Econ Entomol* 9:41–49
- Wolstenholme AJ (2012) Glutamate-gated Chloride channels. *J Biol Chem* 287:40232–40238
- Wolstenholme AJ, Rogers AT (2006) Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. *Parasitology* 131:85–95

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