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Original Article

NON-TARGET IMPACTS OF FIPRONIL BAIT USING MORPHOLOGICAL ALTERATIONS IN THE REPRODUCTIVE SYSTEM OF *BLAPS POLYCRESTA* (COLEOPTERA: TENEBRIONIDAE) AS A BIOMONITOR

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Abstract: Insecticide baits have been successfully used against major groups of urban pests. However, they could affect a wide range of non-target fauna. In Egypt, fipronil bait is a pest control method used for elimination of cockroaches at indoor locations. In the present study, morphological alterations in the reproductive system of both males and females Blaps polycresta (Coleoptera: Tenebrionidae) were used to determine the effects of fipronil bait on beetles as non-target insects. Two groups of insects (control and treated) were used in which, the treated one was exposed to fipronil bait (0.05 %). Treated males showed marked decreases in the size of testes and accessory glands with shrinkage of testicular follicles while treated females exhibited ovarian developmental retardation and ovarian morphological deformations. A highly significant increase was recorded in the number of insects contained abnormalities in both males and females of the treated group as compared with the control one. Mortality was examined in two other groups (control and treated) under the same condition of the morphology test in which, 100 % mortality was achieved after about two weeks for both sexes of the treated group while no mortality was obtained in either sexes of the control one throughout the experiment. The obtained data reinforce the importance of non-target impacts of insecticides and the results support the possible use of morphological alterations in the reproductive system of beetles as a biomonitor of fipronil bait effects.

Key words: Fipronil bait, Coleoptera

INTRODUCTION

Fipronil is a neurotoxic insecticide of the phenylpyrazoles groups. It was discovered and developed by Rhone-Poulenc Agro in 1987 at Ongar, UK [1,2]. It was actively marketed in 1993 as a form of solid (insect bait), liquid spray, or as a granular product throughout a wide range of industrialized and developing countries [3].

Fipronil causes hyper-neural excitation of the central nervous system by blocking two ion channels in the nerves: gamma-aminobutyric acid (GABA) gated and glutamate gated chloride channels [4,5]. Inhibition of GABA, which is an important neurotransmitter in invertebrates, was found to cause hyper excitement, convulsion, and paralysis leading to insect death [3,6].

Fipronil is effective against larval and adult stages of a broad spectrum of insects [7] both by contact and ingestion. It could be delivered via soil, foliar, bait, or seed applications [8]. It is highly effective against cotton boll weevil (*Anthonomus grandis*), *Heliothis* (*Helicoverpa*) *virescens, Spodoptera* spp. and

Alabama argillacea [9]. The use of fipronil as an urban pesticide has been well documented against German cockroach, *Blattella germanica* [10] and some termite species as *Cornitermes cumulans*, *Cornitermes bequaerti* and *Syntermes* sp. [11]. Fipronil is also used in control of mosquito larvae [12,13] and ants [14]. According to a provisional Hazard ranking, fipronil is the most hazardous of six conventional insecticides used in locust control [15].

In Egypt, fipronil essentially controls cockroaches. Insecticide baits have been successfully deployed against major groups of urban pests [16-19]. They have largely displaced other formulations for controlling insect pests as they are much less translocatable, less hazardous, safer and environmentally friendly than insecticide sprays [20]. However, toxic baits could be attractive to a wide range of animals and may kill non-target fauna, especially other invertebrates [21]. Hence, non-target impacts are often a serious concern in eradication programs. As non-target insects were found to be affected by fipronil [22] so, the undesirable effects on these insects are attracting more and more attention [23-25].

Coleoptera as one of the non-target groups was adversely affected by fipronil. Even at very low doses, it causes significant adverse impacts on them (certain Carabidae, Scarabaeidae and Tenebrionidae) reducing their relative abundance [26]. Laboratory toxicity tests of fipronil is well documented in some insect species of Coleoptera (Tenebrionidae) providing a rapid indication of its acute hazard. For example, more than 99 % mortality at the higher doses and 85 % mortality at the lowest dose were obtained with the detritivorous darkling beetles [27].

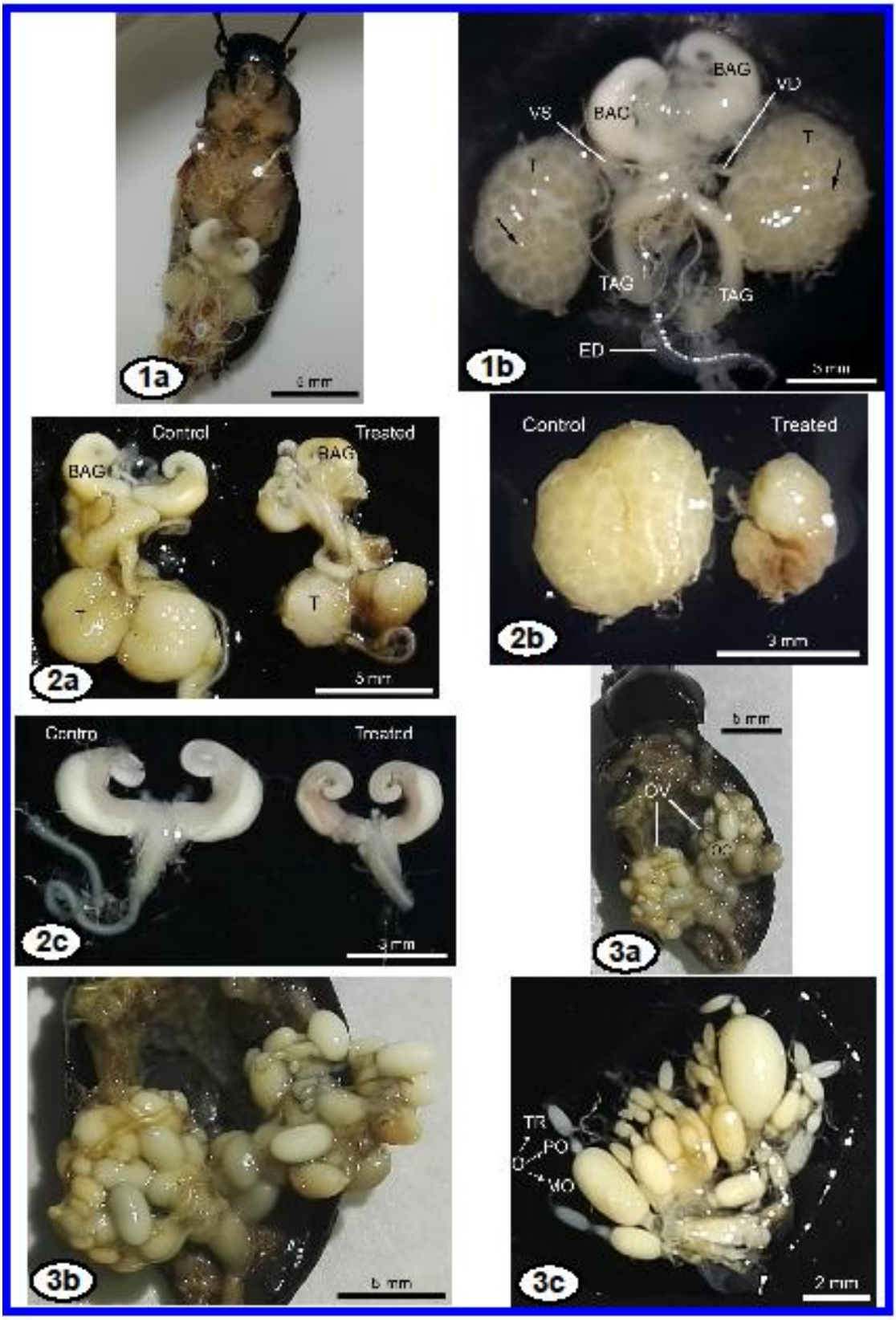
Most studies of fipronil on non-target insects evaluate its toxicological effects [22,26,27]. However, other effects of fipronil on terrestrial invertebrates are scanty, e.g. the adverse reproductive effects on honey bee males [28,29]. Hence, more efforts should be dispensed by using other tools as morphological alterations in different organs of insects. Reproductive system of insects is an important system in studying exposure to pesticides. Morphological studies of the organs that are involved in the production of offspring, associated with toxicological bioassays are of great importance for elucidating the action of these compounds on insects. They could provide information about how these organisms are responding to disturbances of contaminated ecosystems. Hence, morphological evaluation of reproductive system can reveals changes induced by toxicants.

Surprisingly, no studies have investigated morphological effects of fipronil on the reproductive system of non-target insects. As beetles could be used as bioindicators of environmental stresses [30-32], therefore, *Blaps* species (Coleoptera: Tenebrionidae) are good objects of toxicological studies and any disturbance within their reproductive system may lead to important sub-lethal changes of the insect's physiology and behavior. The aim of present study is to evaluate morphological alterations in the reproductive system of *Blaps polycresta* in addition to percentage mortalities in this insect as a non–target insect exposed to fipronil bait.

MATERIAL AND METHODS

The insect sampling: Coleopterous adult insects were collected from the garden of Faculty of Science, Alexandria University, Moharram Bey, Alexandria, Egypt. This garden does not subject to any pesticides or industrial contaminations as the cultivated plants at this site are mostly ornamental plants, grasses, and shrubs [31]. Also, the collected insects have never been subjected to any type of insecticide at any stage of their life. The coleopterous insects were collected from soil, sexed, and counted; then, they were transferred to the laboratory. The insects were identified for their species as *B. polycresta*.

Experimental set-up (Morphology groups): The beetles were maintained in plastic cages at room temperature in laboratory. Eighty adult insects were divided into two groups, each of forty insects (twenty for each sex). The first group (control group) did not receive any types of chemicals and was housed at normal environmental conditions. The second group (treated group) was exposed to fipronil bait that was put to the corners of each insect cage so as to mimic environmental condition available for German cockroach control. A fipronil 0.05 % bait was purchased from the market that is packaged in a childproof plastic container (5 × 5 × 1 cm) and was applied directly to the insects without dilution. On the fourth day of the experiment, the insects of the two groups were dissected in insect Ringer solution in which, reproductive systems of males and females were examined for the presence of morphological alterations and photographed. Numbers of insects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contained abnormalities in their reproductive systems were calculated for both sexes of the two groups throughout the experiment and used in statistical analysis.

Mortality groups: Another two groups (control and treated) of the same numbers of insects and under the same condition of the morphology test were constructed to estimate the mortality of males and females. Numbers of dead insects for each sex were recorded every day till the end of the experiment and percentage mortalities were estimated.

Statistical analysis: For both morphology and mortality groups, laboratory experiments were replicated four times with the same number of insects per each replication. For the morphology groups, test of significant between the control and the treated insects was estimated by Student *t*-test at *p*d" 0.001 using IBM SPSS software package version 20.0 and the results were summarized in a table. For the mortality groups percentages of dead insect were estimated at the end of the experiment.

RESULTS

Normal reproductive system of control male: In control adult male *B. polycresta,* the reproductive system (Figs. 1a, 1b) consists mainly of two biconvex testes, two vasa deferentia, two vesiculae seminalis, an ejaculatory duct and two pairs of male accessory glands. One pair of the accessory glands was coiled and named bean-shaped accessory glands and the other pair named tubular accessory glands (Fig. 1b) as recorded for the beetle, *Tenebrio malitor* [33]. The reproductive system appeared with normal structure and size and in its normal position in the abdomen beneath the alimentary canal (Fig. 1a). Each testis of the control insects is surrounded by fat lobules maintaining them in their normal position and composed of a number of testicular follicles with normal shape and size and without any signs of abnormalities in addition to normal shape and size of accessory glands (Fig. 1b).

Malformed reproductive system of treated male: Reproductive system of treated adult males exhibited deformations in their external structure and a marked decrease in the size of testes with shrinkage of testicular follicles (Figs. 2a, 2b) in addition to a decrease in the size of accessory glands (Figs. 2a, 2c) that was more pronounced in bean-shaped accessory glands as compared with those of the control ones in almost all the treated males.

Normal reproductive system of control female: Reproductive system of control adult female *B. polycresta*, (Figs. 3a,3 b) was described earlier by Osman and Shonouda [32] in which it consists mainly of two ovaries contained large numbers of oocytes, two lateral oviducts, a common oviduct, a vagina and a spermatheca. Each ovary (Fig. 3c) is of telotrophic meroistic type comprising numbers of ovarioles that are enclosed in a connective tissue coat. Each ovariole consists of two parts, the proximal germarium, contains the trophocytes, and the distal vitellarium, comprises follicles in progressive stages of maturation with pro- and mature oocytes (Fig. 3c). Ovareis appeared normal and were fully developed with normal shape and size of ovarioles containing oocytes at different maturation levels but most are fully mature (Figs. 3a,3b,3c). The fully developed mature ovaries appeared with no signs of abnormalities.

Malformed reproductive system of treated female: Reproductive system of treated adult females exhibited a marked decrease in ovarian sizes as compared with those of the control ones (Fig. 4a). Deformations appeared in one ovary (right or left)

Fig. 1a,b: Photographs of control adult males *B. polycresta* showing (**1a)** a whole mount dissected male having normal structure and size of the reproductive system; (**1b)** the structure of a dissected reproductive system with two testes (T) composed of a number of normal testicular follicles (arrows) with normal shape and size and without any signs of abnormalities in addition to normal shape and size of two pairs of accessory glands, bean-shaped accessory glands (BAG) and tubular accessory glands (TAG).Vas deference (VD), vesicula seminalis (VS), ejaculatory duct (ED).

Fig. 2a,b,c: (**2a)** The structure of control and treated reproductive system of adult males *B. polycresta* showing the difference in sizes of testes (T) and bean-shaped accessory glands (BAG); (**2b)** the difference in sizes of control and treated testes; **(2c)** the difference in sizes of control and treated bean-shaped accessory glands.

Fig. 3a,b,c: Photographs of control adult females *B. polycresta* showing (**3a)** a whole mount dissected female having normal structure and size of the reproductive system with two ovaries (OV) appeared mature with mature oocytes (OC), (**3b)** higher magnification of (**a); (3c)** the structure of a normal ovary with normal ovarioles (O) composed of germarium represented by tropharium (TR) and vitellarium having a pro-oocyte (PO) and one mature oocyte (MO).

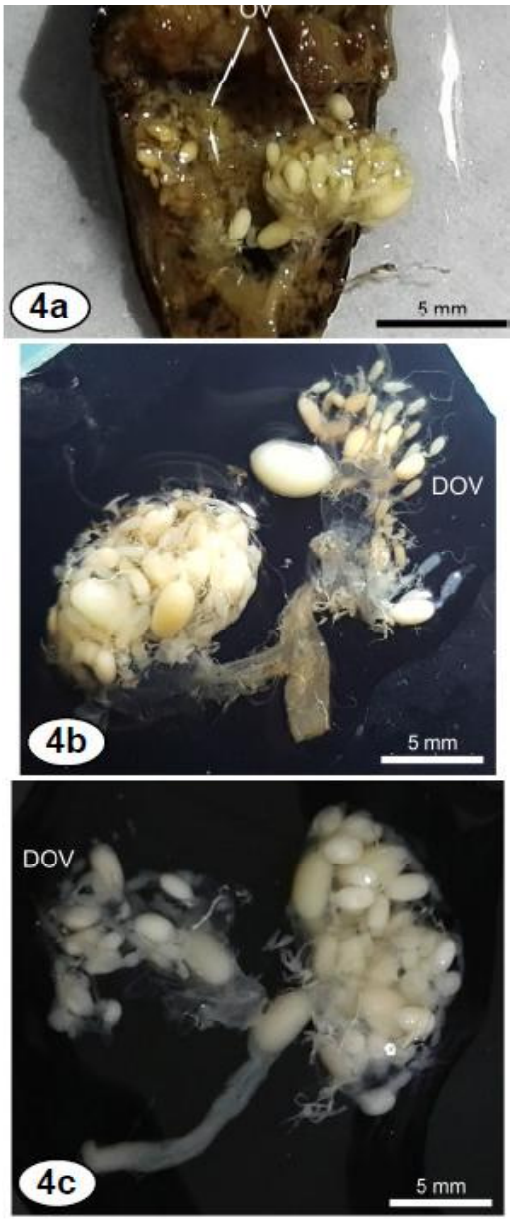

Fig. 4: (**a -c)** Photographs of abnormal treated adult females *B. polycresta* showing (4**a)** a whole mount dissected female having abnormal reproductive system with mature abnormal small sized ovaries (OV) with atrophied ovarioles, (4**b)** abnormal reproductive system with right deformed ovary (DOV), (4**c)** abnormal reproductive system with left deformed ovary (DOV).

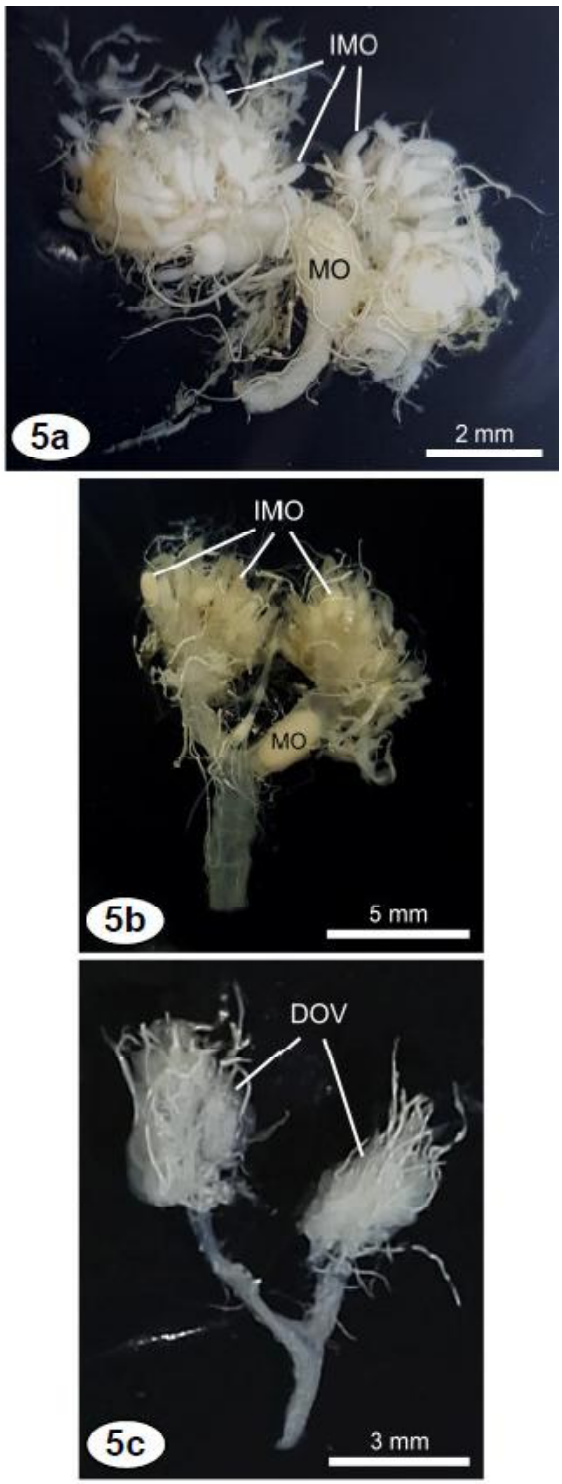

Fig. 5a,b,c: Photographs of abnormal reproductive system of treated adult females *B. polycresta* showing deformations in the two ovaries with small sized immature oocytes (IMO) in (**5a, b)** with only one mature (MO) in the lateral oviduct, (**5c)** complete deformed ovaries (DOV) without oocytes.

(Figs. 4b, 4c) or in the two ovaries (Figs. 5a,5b,5c) with atrophied ovarioles containing few or no vitellogenic egg chambers. The ovariole number is diminished in which, degenerated oocytes can be observed with small numbers of mature ones (Figs.

4b, 4c). Most ovaries from the treated females did not contain large oocytes and in some cases, both ovaries contained a few ovarioles with small sized immature oocytes in the last stages of development with only one mature near the lateral oviduct (Figs. 5a,5b). The mature oocytes of the treated females appeared small in size as compared with the mature ones of the control females. Ovarian developmental retardation, represented as deformed ovaries, with no oocytes (Fig. 5c) was detected in most females of the treated group.

Number of abnormalities in reproductive system of insects in the morphology groups: The number of abnormalities (shape and/or size) in the reproductive system of males and females of the control as well as the treated groups were estimated throughout the experiment (Table 1). Control group showed no morphological alterations in the reproductive system of both sexes (except for one or two insects in four replications). In the treated group, the results pointed out a highly significant increase in the number of insects contained abnormalities in both males and females as compared with the control one (*p*d" 0.001) (Table 1) indicating pronounced effects of fipronil bait treatment on the reproductive system of insects. No significant differences were observed in the number of insects contained abnormalities between males and females of the treated group.

Percentage mortalities of insects in the mortality groups: In addition to the abnormalities obtained throughout the experiment, death of insects was also observed as a final effect of fipronil bait treatment. This was observed in the control and treated insects of the mortality groups in which, the number and percentage mortalities were calculated every day from the beginning of the experiment. For the treated group, mortality begins at the sixth day for both sexes and reaches 100 % at the twelfth to fifteenth day. No mortalities were obtained in either sexes of the control group throughout the experiment.

Table 1: Number **(**Min – Max, n=20) and mean ± SE of abnormalities in the reproductive system of both males and females *Blaps polycresta* in the control and the treated groups. Data represent four replications for each group. Using Student t-test, *: statistically significant at *p* d" 0.001

	Male		Female	
	Control	Treated	Control	Treated
Min – Max	0 – 2	17 – 18	0 – 2	17 – 19
Mean ± SE	0.75 ± 0.48	17.50* ± 0.29	0.75 ± 0.48	18.25* ± 0.48

DISCUSSION

To the authors' knowledge, this is the first evidence of morphological alterations induced by fipronil bait on the reproductive system of insects, especially non-target insects. Hence, the results of the present research could add information about the process of adaptation in organs of insects. The results demonstrated that both males and females of the treated group exhibited deformations in their reproductive systems that appeared highly significant in their numbers as compared with those of the control group.

The effects of fipronil bait on insect's reproductive systems, as observed in the present study, are likely to be due to the disruption of hormonal processes, directly or indirectly controlled by ecdysteroids. In adult insects, ecdysteroids from the follicle cells of testes and ovaries are involved in the maturation and functioning of male and female reproductive organs [34]. Production of smaller ovaries or eggs was reported in the literature as possible inhibitory effects of non-steroidal ecdysteroids in several insect species [35]. The female gonads are the site of synthesis of several hormones that impact insect physiology such as insulin like peptides and ecdysone [36-38]. In addition, vitellogenin synthesis is necessary for the late stage of oogenesis in insects [39]. Hence, fipronil may cause hormonal disturbance inhibiting egg production and disrupting vitellogenin synthesis via the ecdysteroid receptor protein complex. Insufficient concentrations of vitellogenin may then prevent protein synthesis and yolk deposition in the developing oocyte [40,41] and consequently, deformities in the ovaries of treated insects.

Neurotoxic, hepatotoxic, reproductive, and cytotoxic effects of fipronil on vertebrates and invertebrates are well documented in which oxidative stress might play critical roles in its toxicity [42-46] as well as in a large number of biological responses [47]. Fipronil is known to impair oxidative phosphorylation in mitochondria [47] which is essential for spermatozoa functionality [29] hence, may cause abnormalities in male insects. In this respect, more work is required to explain the histological and ultrastructural effects of fipronil on reproductive organs.

Male accessory glands are important organs in the reproductive system of male insects as they act with their proteins as key modulators of reproductive success by influencing the female reproductive

physiology and behavior [48-50]. The present results indicated that male accessory glands in fipronil treated males exhibited marked decrease in their sizes especially the sizes of bean-shaped accessory glands which may be due to the effect of fipronil on the secreted proteins of the glands that could affect the reproductive success of the insects. Moreover, Hentze et al. [51] indicated that tubular accessory glands of *Tribolium castaneum* are the site of ecdysteroid production in the male reproductive system.

The results pointed out that mortality begins at the sixth day of the experiment and reaches 100 % after about two weeks. Fipronil is an active molecule that disrupts the insect central nervous system. Acute and chronic toxicity of fipronil have been discussed by Tingle et al. [3] with a review of the environmental fate of the insecticide in which they reported that death of insects results from the uncontrolled central nervous system activity. Again, Wang et al. [47] reported that oxidative stress plays an important role in cell signaling pathways thus; significant changes in the cell cycle, and the stimulation or inhibition of signal transduction usually result in many toxicological effects. Exposure to fipronil might mediate apoptosis via the generation of ROS and through the oxidative stress-related pathway [47,52].

Given the data of the present work, risk assessment procedures for fipronil and other insecticides need to consider morphological alterations in the reproductive organs of non-target insects. As insecticide baits can have broad adverse effects not only on target pests but also on non-target organisms hence, increasing bait selectivity is of great importance to limit non-target effects [53]. Moreover, insect-specific baits must be placed in desired locations to more effectively target the pest [19].

CONCLUSIONS

The results demonstrated that both males and females of the treated group exhibited deformations in their reproductive systems with marked decrease in their gonads as compared with those of the control ones. Shrinkage of testicular follicles of treated males with marked decrease in the size of testes and male accessory glands as well as developmental retardation and morphological deformations in ovaries of treated females were the most detected alterations in the treated group. Hence, the reproductive systems of treated males and females showed poorly defined stages of testicular and ovarian development while control insects showed normal development of their reproductive systems represented by normal size and maturation of adult gonads. One-hundred percent mortality of the treated insects was recorded after about two weeks of the experiment compared with 0 % mortality of the control ones. The obtained data reinforce the importance of non-target impacts of insecticides and the results support the possible use of morphological alterations in the reproductive system of beetles as a biomonitor of fipronil bait effects. Hence, reproductive disorders induced by fipronil and other insecticides must be taken into consideration in the assessment of pesticide risks. Responses of the present insect to toxicity and adaptive mechanisms to stress induced by exposure to fipronil bait must be investigated which will be the subject of the future work.

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