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Toxic and infertility effect of spirotetramat compound against male black rat *Rattus rattus* (Rodentia: Muridae) under laboratory and field conditions

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Abstract

The efficiency of spirotetramat bait against black rat, *Rattus rattus* (L.) (Rodentia: Muridae) was studied in this work. The impact was conducted under laboratory (non-and free choice feeding methods) and field conditions. In the non-choice feeding technique, serial concentrations of spirotetramat as bait were offered to rats during different periods to evaluate the most effective bait concentration that causes a high mortality rate. Additionally, free-choice feeding tests play important role in estimating palatability. Furthermore, the application of spirotetramat in citrus trees was occurred to evaluate the efficiency on reduction of rat population. Our results depicted that the most effective bait concentration which achieves 100% mortality was 1.4 % and the time of death ranged between 4-10 days with 3.4 days mean in non-choice method. Using a free-choice technique produced 41.6 % acceptance as well as 80 % mortality and the time of death ranged between 5-12 days with 6.4 days mean. The toxic action of the tested compound was investigated *via* detection of some biochemical and histological changes. Feeding on spirotetramat treated bait (0.9 %) for 7 days induced a significant decrease in serum total lipids, total protein levels as well as a significant increase in both brain and testis lipid peroxides. Regarding male hormone levels, feeding obtained a remarkable suppression of testosterone, FSH and LH levels. Spirotetramat bait toxic effect in this study is supported by the histopathological alterations in both brain and testis tissues. In addition to that, field application revealed achieved 80 % reduction in rat population**.** In conclusion, spirotetramat bait induced lethal action against black rats in the laboratory and field evaluations as well as its effect on the fertility of male rats.

Introduction

Rodents cause economic losses for the agriculture sector, including loss of crop production, food safety concerns, disturbances in animals and poultry farms, disease transmittance and damage to irrigation and water storage infrastructure (Jokić *et al*., 2010 and John, 2014). It developed bait shyness and resistance to the currently used rodenticides (Eason *et al.*, 2010 and Crowell *et al.*, 2013). Spirotetramat, a tetramic acid derivative, is a systemic pesticide

(Nauen *et al*., 2008 and Arnaudov and Petkova, 2020). Spirotetramat inhibits acetyl Co- A carboxylase; a key enzyme in fatty acid biosynthesis (Marcic *et al.*, 2012 and Zangiabadi *et al*., 2019). Also, it is demonstrated acute toxicity in rats *via* oral administration (LD_{50} 2000 mg/kg b.wt) (US APA, 2008). Spirotetramat administration caused 50% and 100% mortality at a dose of 1250 and 1500 mg/kg after 7 days in male Wistar rats (Zangiabadi *et al.*, 2019). Moreover, it can produce severe toxicity through oral consumption in rats as well as motor and balance disorders, dehydration, swelling, decrease in activities and seizure (Australian Pesticides and Veterinary Medicines Authority, 2009). It was reported that pesticides mixtures (Spirotetramat, spiromesifen and cypermethrin) caused a decrease in lipid profiles (Cholesterol, HDL and LDL) and total protein in rats (Sebti and Leghouchi, 2023). Pesticides are known to produce oxidative stress by overproduction of reactive oxygen species and decreasing levels of cellular antioxidants (El-Demerdash, 2011 and Sule *et al*., 2022). Oxidative stress is essential to create a balance between produced free radicals and their metabolism for the appropriate function of testicular cells (Romeo *et al*., 2004). As depicted that pesticide mixtures containing spirotetramat led to a decrease in lipid peroxidaseand antioxidant enzyme activities (SOD, CAT and GPx) in the liver and kidney tissues of Wistar rats (Sebti and Leghouchi, 2023). Oxidative stress is an important factor for development of male infertility because of very high rate of cell division and mitochondrial oxygen consumption in testicular tissue as well as comparably higher levels of unsaturated fatty acids in this tissue than in other tissues (Asadi *et al*., 2017). Oxidative stress causes of male and female infertility including reduction in sperm motility, sperm DNA damage and increased risk of recurrent abortions and genetic diseases (Agarwal *et al*., 2014 and Alahmar, 2019). Reactive oxygen species reduce the male sex hormone; testosterone, FSH and LH levels and disrupt the hormonal balance that regulates male reproductive functions (Saylam and Cayan, 2020). Non- and free choice feeding methods are used to determine mortality rates and acceptance of pesticides (El-Abd *et al*., 2022 and Kandil *et al*., 2022).

The main aim of this work is to study toxicity of spirotetramat as bait under laboratory and field conditions and its direct effect on the reproductive system of adult male black rats *Rattus rattus* (L.) (Rodentia: Muridae) to use in rodent pest management programs.

Material and methods

1. Tested compound:

1.1. Common name: Spirotetramat insecticide: carbonic acid, cis-3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro [4.5] dec-3-en-4-yl ethyl ester; BYI 8330.

1.2. Trade name: Movento (10% SC). The oral LD_{50} value for rats is 2000 mg/kg b.wt. (US APA, 2008)**.** It was obtained from Bayer A.G. Company, Germany. It was used as a bait, mixed with crushed maize at different concentrations.

2. Laboratory evaluations:

2.1. Experimental animals:

The adult individuals of male black rats, *R. rattus*, were caught by rat traps (30×15×20 cm) from fields and stores located in Kerdasa, Giza, then transferred to the Harmful Animals Research Laboratory, Plant Protection Research Institute, Agricultural Research Center (ARC), Dokki, Giza, Egypt. Rats were adapted individually in cages of size (50×30×30 cm) and fed on crushed maize and water at 20-25°C and 12 hrs. daily light, dark cycles for 15 days before the beginning of the

experiments. Twelve groups (Ten rats for each) of healthy rats (180-200 g/kg b.wt) were used in this study. These groups were differentiated into 8 groups for non-choice method (4 groups for 3 days and 4 groups for 7 days). One group of rats was used for free-choice feeding method. Other three groups were used as controls.

2.2. Non-choice feeding test:

Serial concentrations of spirotetramat bait (0.4, 0.6, 0.9, and 1.4 %) by a constant factor of (1.5) were tested using non-choice feeding technique to find out the best concentration that realized the highest mortality rate. Fifty grams of treated bait were offered to each rat for different periods (Four groups for 3 days and others for 7 days), and the two control groups were fed at 50 g of crushed maize. The treated and untreated bait was renewed daily during the treatment period and the consumed amount was daily estimated. The treated bait was taken out and the surviving animals were fed on a diet and observed up to 28 days. The mortality was recorded (Shefte *et al*., 1982).

2.3. Free -choice feeding test:

Free-choice feeding test is a vital method for evaluation of the palatability of spirotetramat bait (1.4%) comparing with the rats consumption of challenge diet (65% crushed maize + 25% ground wheat + 5% sugar + 5% corn oil) according to Palmateer (1974). The treated bait and challenge diet were presented to each rat (50g for each) in small separate tureen for 7 days. Their positions were daily changed for avoiding feeding preferences for a specific location. The consumed amount of bait and diet were recorded daily. The mortality percent was recorded. The bait acceptance percent was recorded using the following equation (Mason *et al*., 1989). Untreated rats group was fed on the standard diet.

$$\text{Acceptance \%} = \frac{\text{Consumed amount of treatment bait (g)}}{\text{Consumed amount of treatment bait (g) + challenge diet (g)}} \times 100$$

3. Biochemical and histopathological investigations:

3.1. Samples preparation:

For biochemical and histological assessments, the survived animals which fed on 0.9 % treated bait for 7 days were sacrificed with diethyl ether anesthesia. Blood and tissue samples were collected. Part of the brain and one testis were separated, placed in Na Cl (0.9%) and were homogenized by Teflon homogenizer under cooling. The samples were centrifuged at 3000 rpm for 10 min. The clear supernatant serum was removed and kept in a deep freezer at -20ºC until used. Blood and tissue samples were collected by the same process.

3.2. Biochemicals assessment:

Serum total lipids, brain and testis lipid peroxides levels were assessed utilizing a reagent kit bought from Biodiagnostic Co. (Egypt) according to Zöllner and Kirsch (1962) and Ohkawa *et al*. (1979), respectively. Serum total protein was estimated according to Titez (1994) using kits from Spectrum Co. Serum testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were estimated by rat enzyme-linked immunosorbent assay (ELISA) commercial kits purchased from Cusabio Biotech CO., Wuhan, China, according to the manufacturer's protocol.

3.3. Histopathological screening:

After dissection brain and one testis from each rat were rapidly removed and fixed in 10% neutral buffered formalin for 24 hrs. Then, they were washed in running tap water and serial dilutions of ethanol were used for dehydration process, cleared in xylene,

then embedded in paraffin at 56°C in hot air oven for 24 hrs. The paraffin wax tissue blocks were prepared for sectioning by microtome at 4 µm thickness. Freshly prepared sections, floating on a 40°C water bath containing distilled water, were collected on glass slides, deparaffinized, stained with hematoxylin and eosin (H&E) stains according to the method of Banchroft *et al*. (1996).

4. Field application:

Spirotetramat bait (1.4%) was evaluated under field conditions of Abu-Rawash, Giza Governorate that was infected with *R. rattus*. The area of 2000 m^2 of citrus trees was divided into three regions for treatment and three as control. Using the food consumption method, the population density of rats was assessed pre and post treatment and this method is according to Dubock (1984). Pre-treated with crushed maize 3000 g (In small plastic sacks 250 gm of each) was put inside bait stations which distributed in the field. The consumed amount of food was calculated for only the fourth and the fifth day. After that treated bait was applied and renewed every 3 days until consumption stopped. For one week, the bait stations (Figure 1) were left empty. Then untreated crushed maize was placed inside each bait station for five days. The consumption was recorded and the rat population reduction was calculated as follows:

$$\text{Population reduction \%} = \frac{\text{Pre-treatment consumption (g) - post-treatment consumption (g)}}{\text{Pre-treatment consumption}} \times 100$$

Figure (1): Bait station for field application.

5. Statistical analysis:

The experimental design was completely randomized with different replicate. The obtained data were statistically analyzed by one way ANOVA and Least Significant Difference (LSD) at ($p \leq 0.05$) using the COSTAT program (Glenn, 2005).

Results and discussion

1. Effects of spirotetramat bait against *Rattus rattus* using non-choice and free- choice feeding technique:

Data in Table (1) demonstrate the effect of serial concentrations of spirotetramat bait against male *R. rattus* using non-choice feeding technique to cause the highest mortality percent. A gradual increase in mortality rate was depicted with increasing the compound concentration and increasing time of feeding. Concerning the tested concentrations, (0.4, 0.6, 0.9 and 1.4 %) caused 0, 20, 40 and 60% mortality percentages, respectively with the average consumption 10.28, 9.80, 9.30 and 8.36 g, respectively. The time of death ranged between 0-0, 9-13, 6-14 and 5-12 days with mean 0, 11, 7, 25 and 7 days, respectively when rats were fed for 3 days. Increasing feeding time with spirotetramat bait for seven days increased the mortality percent to be (20, 40, 70 and 100 %) with average consumption 9.56, 8.70, 7.98 and 6.9 g. The time of death ranged between 7-10, 6-13, 6-13 and 4-10 days with mean 8.5, 9.5, 6 and 3.4 days, respectively. The highest concentration of bait (1.4 %) induced complete morality (100%) with an average consumption of bait 6.9 g compared with 10.56 g for control. Feeding decreased in bait-treated rats with an increasing time period. There was a significant decrease in treated bait consumption compared to untreated rat feeding.

Table (1): Effect of different concentrations of spirotetramat bait against black rat, *Rattus rattus*, using non- choice feeding technique.

Feeding Periods / Bait concentration (%)	3 days						7 days					
	Average consumption (g) (Mean±SE)		LSD	Mortality %	Time of death (day)		Average consumption (g) (Mean±SE)		LSD	Mortality %	Time of death (day)	
	Treatments	Control			Range	Mean	Treatments	Control			Range	Mean
0.4	10.28^{ab}±0.18	10.40^{a}±0.17	0.446	Zero	.....	……	9.56^{b}±0.20	10.54^{a}±0.18	0.598	20	7-10	8.5
0.6	9.80^{b}±0.12			20	9-13	11	8.70^{c}±0.09			40	6-13	9.5
0.9	9.30^{c}±0.05			40	6-14	7.25	7.98^{d}±0.14			70	6-13	6
1.4	8.36^{d}±0.19			60	5-12	7	6.9^{e}±0.32			100	4-10	3.4

Values are expressed as means (consumptions) ± standard errors.

^{abcd} values in column with different letters are significantly different at (P ≤ 0.05). LSD: Least Significant Difference.

Regarding the free-choice feeding test with spirotetramat bait (1.4%) in Table 2, the average consumption of challenge diet was 7.06 g, but was 5.02 g for treated bait compared with the average consumption of control rats was 10.74 g. There was a significant decrease in treated bait and challenge diet compared with control rats. The treated bait produced high acceptance percent 41.6 % as well as 80 % mortality and the time of death ranged between 5-12 days with 6.4 days mean.

Table (2): Effect of spirotetramat bait (1.4%) against black rat, *Rattus rattus via* free- choice feeding technique.

Average consumption (g) (Mean±SE)			LSD	Acceptance %	Mortality %	Time of death (day)	
Control	Challenge diet	Treated bait				Range	Mean
10.74^{a}±0.19	7.06^{b}±0.20	5.02^{c}±0.18	0.451	41.6	80	5-12	6.4

Values are expressed as means (consumptions) ± standard errors.

^{abc} values in column with different letters are significantly different at (P ≤ 0.05). LSD: Least Significant Difference.

2. Biochemical assessment:

Table (3) shows the effects of 0.9 % treated bait on male rats after 28 days of treatment. Feeding induced significant decrease in serum total lipids and total protein levels with difference percent of -30.14 and -32.17 % compared with control rats. Moreover, this concentration resulted in an increase in lipid peroxide activity in both brain and testis with difference percent of 27.27 and 153.39 %, respectively compared with untreated rats. Results tabulated in Table (4) show the effect of spirotetramat treated bait on some hormones of *R. rattus.* Serum levels of testosterone, LH and FSH were decreased significantly with difference percent of -26.92, -19.79 and -7.74 %, respectively compared with controls.

Table (3): Effect of spirotetramat bait (0.9 %) after feeding for seven days on some biochemical parameters of *Rattus rattus*..

Parameter	Control	Treatment	Difference %	LSD
Total Lipids (mg/dl)	365^{a}±22.94	255^{b}±2.50	-30.14	63.98
Total Protein (mg/dl)	10.57^{a}±0.41	7.17^{b}±0.38	-32.17	1.54
Brain lipid peroxides (nm/g. tissue)	50.54^{b}±1.34	64.32^{a}±3.82	27.27	11.22
Testis lipid peroxides (nm/g. tissue)	6.93^{b}±0.60	17.56^{a}±0.55	153.39	2.27

Values are expressed as means ± standard errors.

^{ab} values in column with different letters are significantly different at (P ≤ 0.05). LSD: Least Significant Difference.

Table (4): Effect of spirotetramat bait (0.9 %) after seven days feeding on some hormones of male *Rattus rattus*..

Hormones	Control	Treatment	Difference %	LSD
Testosterone (ng/ml)	0.52^{a}±0.02	0.38^{b}±0.04	-26.92	0.126
LH (mIU/ml)	0.96^{a}±0.03	0.77^{b}±0.01	-19.79	0.085
FSH (mIU/ml)	1.55^{a}±0.03	1.43^{b}±0.03	-7.74	0.108

Values are expressed as means ± standard errors.

^{ab} values in column with different letters are significantly different at (P ≤ 0.05). LSD: Least Significant Difference.

3. Histopathological investigations:

As depicted in Figure (3), feeding with 0.9 % treated bait induced alterations in brain tissue in the form of necrosis in neurons compared with the normal histoarchitecture in untreated rats Figure (2). As well as, treatment caused various lesions in testis tissue including lose of spermatogonic series from the lumen of seminiferous tubules

in Figure (5) and coagulation of the lumenal content of seminiferous tubules in Figure (6). On the other hand, normal spermatogonial cells and sperms in untreated rats were observed in Figure (4).

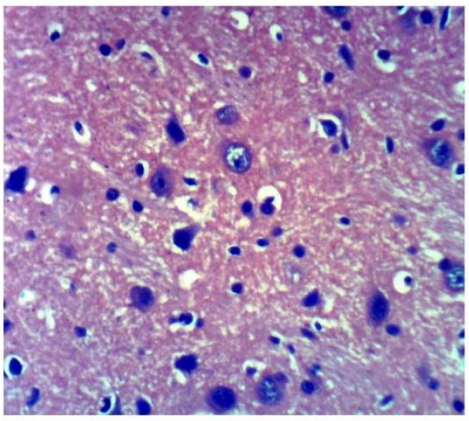

Figure 2: Photomicrograph of H and E stained brain section of untreated rats showing no pathological changes. x 400

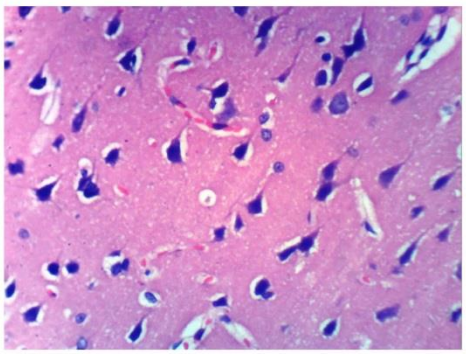

Figure 3: Photomicrograph of H and E stained brain section of spirotetramat-treated rats showing necrosis of neurons. x 400

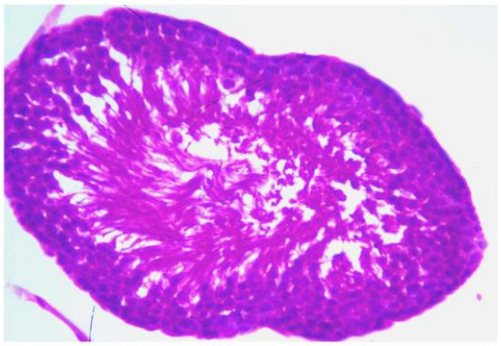

Figure 4: Photomicrograph of H and E stained testis section of untreated rats showing normal structure of testis; normal spermatogonia, spermatid and spermatozoa. x 400

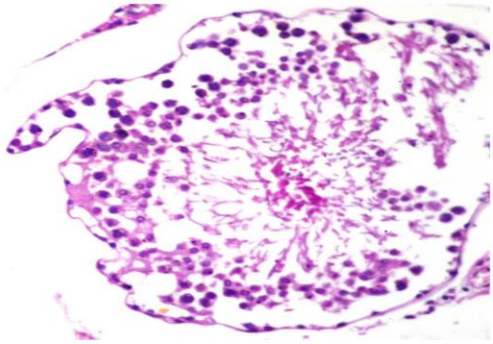

Figure 5: Photomicrograph of H and E stained testis section of spirotetramat-treated rats showing lose of spermatogonic series from the lumen of seminiferous tubules. x 400

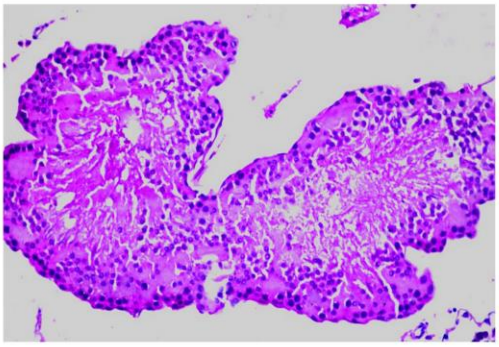

Figure 6: Photomicrograph of H and E stained testis section of spirotetramat-treated rats showing lose of spermatogonial cells and sperms with coagulation of the lumenal content of seminiferous tubules. x 400

4. Field implementation:

The efficiency of spirotetramat bait (1.4%) was evaluated against *R. rattus* in citrus trees as shown in Table (5). The average consumption of crushed maize in pre-treatment period was 1250.33 g while the consumption of post-treatment was 250 g and treated bait consumption was 1019.00 g. The data demonstrated that spirotetramat bait achieved 80 % reduction in rat population. There was a significant difference at ($p< 0.05$) between average rat consumptions during the experiment period.

Table (5): Efficiency of spirotetramat bait (1.4 %) against *Rattus rattus* under field conditions.

Average consumption of bait (g) Mean ± SE				Population reduction %	LSD
Pre-Treatment	Treatment	Post-Treatment	Control	80	111.20
1250.33[b] ±40.46	1019.00[c] ±26.70	250[d] ±40.46	1805.27[a] ±25.97		

Values are expressed as means (consumptions) ± standard error.
[abcd] values in column with different letters are significantly different at (P ≤ 0.05). LSD: Least Significant Difference.

In our study, treatment of rats with different concentrations of spirotetramat using non-choice technique caused death. Furthermore, the mortality ratio increased with increasing the concentration of treated bait and this may be due to the toxic impact of the tested bait inducing disturbance in body function. So, rat body became unable to combat the tested compound. Moreover, by using free-choice technique, rats were not averse to the treated bait and this may be due to the high acceptability of spirotetramat bait. These results agree with (Kandil *et al*., 2015 and Kandil *et al*., 2022), who reported that various insecticides have rodenticidal efficacy in laboratory and field experiments. Lipids are required for synthesis of amino acids, formation of cell membranes, hormonal regulation and proper reproductive health (Jones and Rideout, 2014). Reduction in total lipid maybe due to symptoms caused by spirotetramat bait that inhibits the acetyl coenzyme- A- carboxylase. This data agrees with (Bruning *et al*., 2018) who recorded acetyl-co-A carboxylase plays a major role in fatty acid synthesis, so this enzyme is the hub of the fatty acid synthesis-related metabolism network. Spirotetramat inhibits lipid biosynthesis and may cause dysfunction in biological and histological processes within wild rats (Marcic *et al*., 2012 and Zangiabadi *et al*., 2019). Synthesis of fatty acids controls the expenditure and storage of carbon sources and energy that can regulate other metabolic pathways, such as glucose and amino acid metabolism (Currie *et al*., 2013; Hodson and Gunn, 2019 and Imamura *et al*., 2020). Proteins play an important role in building muscles, hormonal and all body tissue. Concerning this work, decreasing in total protein may be due to toxic effect of spirotetramat on amino acids, which are essential for protein synthesis and decrease of fatty acid.vis / versa results occur with (Xu *et al*., 2022) who proved that chemosensory protein exhibited stronger resistance to spirotetramte in some organisms due to play a defensive role of protein within the body.

Reduction of protein caused increase fat accumulation in Wistar rats (de Oliveira *et al*., 2013 and Malta *et al*., 2014). In the present study, feeding with spirotetramat treated bait promoted increases in both brain and testis lipid peroxide activities. This elevation is supported by the tissue alterations in the form of necrosis of

neurons in brain tissue as well as various lesions in testis tissue, including lose of spermatogonic series from the lumen of seminiferous tubules and coagulation of the lumenal content of the seminiferous tubules. This finding is consistent with Sebti and Leghouchi (2023) who revealed that a pesticide mixture containing spirotetramat induced remarkable increase in lipid peroxide level of rats. El-Demerdash (2011) mentioned that pesticides are known to induce oxidative stress by increasing reactive oxygen species generation and reduction levels of cellular antioxidants. As these radicals produced in excess, a phenomenon called oxidative stress is generated. This process plays a central part in the development of many diseases (Srivastava and Kumar, 2015; Aminjan *et al*., 2019 and Tsatsakis *et al*., 2019). As mentioned by Sule *et al*. (2022) that the formation of oxygen free radicals can be regarded as a major factor in the toxicity of various pesticides. So, development of male infertility depends on oxidative stress. Because increasing of reactive oxygen species is an important factor for cell division and mitochondrial oxygen consumption in testicular tissue (Asadi *et al*., 2017). Consequently, as a result of treatment with spirotetramat, the testicular biological system fails to detoxify or repair the adverse impact of free radicals leading to tissue damage as reported by (Romeo *et al*., 2004 and Showell *et al*., 2011).

Disturbance in male sex hormones is an indication of reproductive dysfunction. In the current study, feeding on spirotetramat treated bait motivated remarkable decrease in testosterone level and this may be due to the decline in FSH and LH hormone. This deficiency in hormones levels was due to the direct impact of spirotetramte on hormones-secreting gland, damage of testis tissue, as well as increase in free radicals. Our results are in agreement with O´Hara *et al*. (2015) and Asadi *et al*. (2017). Saylam and Cayan (2020) who demonstrate that testosterone level decrease is related to suppression of androgen synthesis and induction of apoptosis in the leyding cells. LH receptors are located on the membrane of the leyding cells, whereas FSH receptors are located on sertoli cells. LH and FSH are combining to synthesis of testosterone (Darbandi *et al*., 2018). The decrease in LH and FSH hormones in this study may be due to increasing in free radicals affecting glands. Our data are in agreement with Saylam and Cayan (2020) who explained that decreasing FSH level reduces the release of androgen binding protein from the cell. As a result of oxidative stress decreases the amount of circulating testosterone. These findings proved testicular toxicity of spirotetramat and these results are in line with US EPA (2008). These impacts give evidence that spirotetramat has antifertility action in rats.

Concerning field application, spirotetramat bait promoted 80 % reduction in rat population. This high efficacy of spirotetramat in reduction of rat population may be related to spirotetramat palatability as well as preference of rats for the bait provided to them and the bait can withstand humidity, light, temperature. This finding is similar with El-Abd *et al*. (2022) and Kandil *et al*. (2022).

This study indicates that spirotetramat has a toxic impact on rats *via* lipid synthesis inhibition, induction of reactive oxygen species led to antifertility effect.

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