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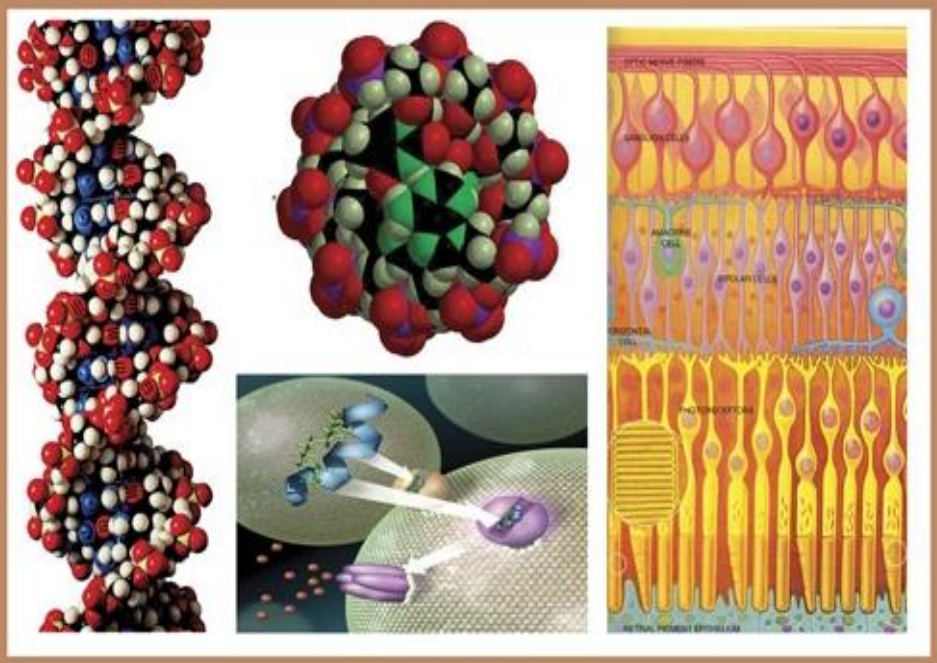

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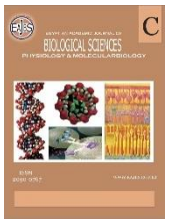

# Effects of Diflubenzuron on Male Fertility in Domestic Rabbit (*Oryctolagus cuniculus*)

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**ABSTRACT**

The aim of the current investigation has been to evaluate the toxic effects of Diflubenzuron (DFB) insecticide on domestic rabbits. In this study, we aimed to evaluate the toxicity of DFB on some reproductive parameters in male *Oryctolagus cuniculus* rabbits. In animals (6 to 7 months and 1700 ± 170 g) the insecticide was used on three different doses:250,500,750 mg/kg. The insecticide was daily administrated for four weeks of experimental periods. Results showed the following effects as compared with the control group: a-decrease in reproductive indicators (concentration, motility, speed, and vitality of spermatozoa) and in serum testosterone concentration. Histological examination showed pathological alterations such as a remarkable reduction in spermatozoa concentration and cellular malformation within seminiferous tubules and epithelial cells especially in groups treated by the highest dose of Diflubenzuron, data demonstrated that DFB caused an effect on reproductive function in adult male rabbits.

## INTRODUCTION

A Pesticide is defined as any substance or combination of substances used to prevent or eradicate unwanted including vectors of diseases in human beings and animals, weeds, fungi, or animals in order to enhance food production and help production processing, storage, transport, or marketing of the food and agricultural commodities (Abdollahi*et al*,.2004 .celementi*et al*,.2008 shadnia *et al*,2005,Sifakis *et al*,.2011) but the use of the sesubstances for a long term caused toxicity on environment (Pogribny Rusyn, 2013) because pesticide residues are found in the food chain, soil and water supplies (Roma *et al*,.2012). Pesticides exposure has increased the concentration of their potential hazard to the health including acute and chronic poisoning (Mostafalou and Adolhali2013 a,b)Moreover it has adverse effects on central nervous, endocrine and reproductive systems (Saadiand Abdolahi2012a,b) heart damage (Vadhana *et al*,.2011). Some pesticides are agents disrupting the endocrine glands leading to change in the normal level of reproductive hormone (Souheila *et al*,.2011, Tabb and Blumberg,2006). Other negative effects of pesticides in male infertility include a disorder in the normal differentiation of chromosomes during meiosis by causing number anumalies in sperm chromosomes (Wasim *et al*,.2009). Pesticides infiltrate the blood circulation and directly head to the production on inferlit sperms by damaging and destroying the cells of the testes tissue or as mutagenic substances in Cells (Larsen *et al*,.1998).

In recent studies, the third lineage insecticide IGR (Insect Growth Regulator) that has minimal effects on the environment and non-target organisms have been used in the control of insect larvae. The IGR insecticides consist of juvenile hormone analogues and a chitin synthesis inhibitor known as diflubenzuron which was discovered by Philips and Duphar Laboratories in the early 1970s (Van Daalen*et al*,.1972; WHO 2008). Diflubenzuron [1- (4-chlorophenyl)-3-(2.6-flurobenzoyl) urea] is individual of benzoylphenylurea groups and has a white crystal structure, Diflubenzuron has been reported to be more larvacidal effect on mosquito larvae rather than on a lot of aquatic organisms, bees, fishes, birds and Mammalia (Karim, 1998; Husien 2006; Maduenko and Martinez, 2008; Marrs, 2012). This pesticide inhibits chitin synthesis during the cuticle occurring and moulting in insect development stages (Reynolds, 1987). Mortality existed in insects either due to the failure of moulting (Ascher and Nemy, 1976; Salokhe *et al*,.2012; Marrs, 2012) or loss of liquid during the moulting (Mulder and Gijswijt, 1973). This study was designed to evaluate the impact of diflubenzuron (DFB) insecticide on some infertility parameters such asthe level of sex hormone (Testosterone) and their effect on some characteristics of sperms and histology of testicular of sperms and histology of testicular tissue in domestic male rabbits *Oryctolagus cuniculus.*

## MATERAILS AND METHODS

### Animals:

Twenty male adult Oryctolagus cuniculus rabbits, aged 6 to 7 months and weighted 1700±130 g were provided from local agricultural fields in Annaba at Algeria. Animals were housed in steel cages (50cm × 60cm ×53 cm) and acclimated for two weeks for adaptation under controlled conditions of temperature (25± 1oC), humidity ( 40 -50 %), and light ( 12h day: 12h night). Food (a mixture of wheat grains, corn, barley, green plants, and carrots) and water were available to add. Rabbits were divided into four groups, the first has five rabbits served as a control, the second (D1, D2, and D3) and the third (D'1, D'2 and D'3) groups of 15 rabbits divided into 3 lots each of 5 and- treated by diflubenzuron at 250g/mg, 500 and 750g/mg by oral gavage respectively for 24 days. Animals were sacrificed by decapitation after the last treatment. The experimental procedures were carried out according to the National Institute of Health guidelines for Animal Care and approved by the Ethics Committee of our Institution.

### Spermatozoa Characterization:

Epididymis were removed and epididymis slits were realized to evaluate the sperm characteristics (movement, concentration, speed, and vitality of the spermatozoa) by taking drops of 1µL of the milky white sperm in 49 µL of physiological fluid (NaCl = 0.9 %) (OMS, 1993) as bellow:

### Sperm Concentration:

Concentration was estimated using a hemocytometer (Malassez slide), counting spermatozoa in 5 squares of the slide by means of the optical microscope (×400) according to the following equation:

Concentration (Spz ×106/ml) = $\frac{D.V.n}{N}$

D (dilution factor),n(number of spermatozoa in 5 squares of the hemocytometer),

V (hemocytometer volume), N (number of squares (100 squares) (OMS, 1993).

### Sperm Motility:

Sperm motility was effectuated on normal slide by identifying 5 areas of vision to count the percentage of moving spermatozoa by optical microscope (×400) (OMS,1993).

### Sperm Speed:

Spermatozoa speed (Speed (µm/s) = distance/time) was determined by placing a drop of semen on Nageottehemocytometer and covered by a cover slip. We calculated the time spent by spermatozoa to cross two parallel lines using a chronometer by optical

microscope (×400) (OMS,1993).

**Sperm Vitality:**

The principle of this test is that living cells do not permit the passage of vital dyes and pigments (Eosin 1%). While dead cells have a plasma membrane that allows the passage of vital dyes and pigments. On a slide, we placed a semen drop, then we deposited eosin 1 % and we mixed the two drops. After drying, the slide was placed under a microscope (×400) and the ratio of colored (dead) and uncolored (living) spermatozoa was calculated (OMS,1993).

**Testosterone Analysis:**

Blood samples were collected into dry tubes, centrifuged at 5000 ppm to obtain serum which served as testosterone concentration measure using a competitive immune-enzymatic colorimetric method (ELISA) (Joshi et al., 1979).

**Histological Analysis:**

Testes were removed and immediately fixed in Bouin solution and processed by using a graded ethanol series, then embedded in paraffin. The paraffin sections were cut into 5 µm thick slices and stained with hematoxylin and eosin for histological examination under light microscopy. The sections were viewed and photographed. (Martoja and Martoja, 1967).

**Statistical Analysis:**

Data are expressed as means ± SD. The significance of the differences in mean values between the control and treated groups was evaluated following the student's-test using Minitab 13 software version 13. Differences were considered statistically significant at $p \leq 0.001$.

## RESULTS

**Spermatozoa Concentration:**

Results showed a very high significant decrease in spermatozoa concentration in treated groups compared to controls (Table 1). This decrease was directly proportional to the concentrations of Diflubenzuron.

**Spermatozoa Motility (%):**

The results shown in (Tables 1) indicated a significant more decrease in mobility in treated rabbits comparing with thecontrol.

**Spermatozoa Speed (µm/s):**

Treatment with Diflubenzuron indicated a very high significant decrease in spermatozoa speed (Table1).

**Spermatozoa Vitality (%):**

Results revealed a significant increase in dead spermatozoa and consequently a significant decrease in living spermatozoa in treated rabbits, with DFB compared to controls (Tables 1).

**Testosterone Concentration:**

Rabbits treated with Diflubenzuronproduced a very low significant decrease in serum testosterone concentrations compared to controls. The reduction was the most in elevated doses of (250 and 500,750 mg /kg) respectively (Figs. 1&2).

**Table 1.**Effect of Diflubenzuron treatment on spermatozoa biological characteristics (concentration, motility, speed, and vitality).

| Reproductive paramètres Groups | | Spermatozoa concentration ($10^6$/mL) | Motility (%) | Speed (µm/s) | Vitality (%) |
|---|---|---|---|---|---|
| | | | | | Alive spz |
| Group 1(T) | | 402±35 | 64±5,77 | 51,56±5,61 | 64±4,18 |
| Groups | $D_1$ | 300,42±37,07*** | 55±7,07*** | 44,50±4,76*** | 55,01±3,15*** |
| | $D_2$ | 222,19±23,10*** | 41,50±4,60*** | 33,40±4,15*** | 42,50±3,39*** |
| | $D_3$ | 120,49±18,15*** | 33,38±14,60*** | 25,32±2,97*** | 37,32±2,15*** |

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

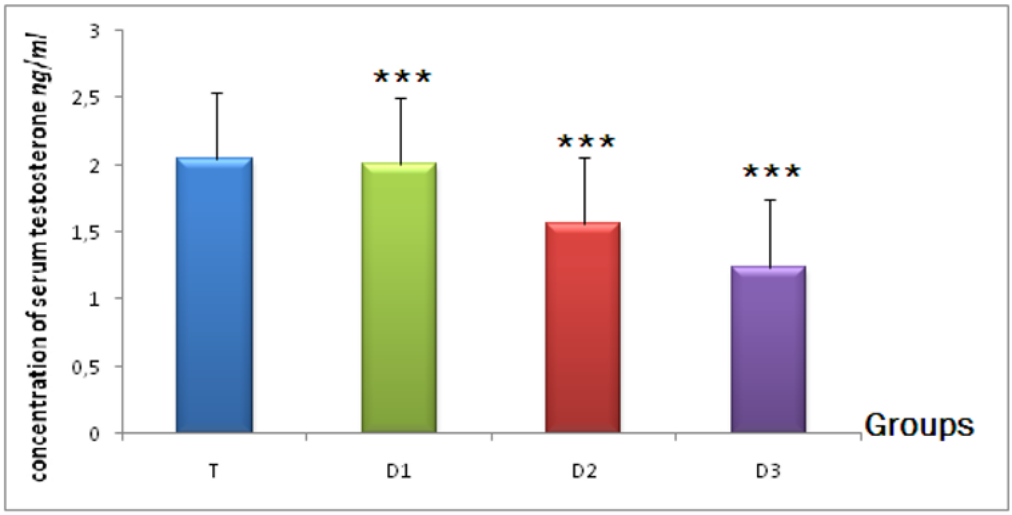


**Fig. 1**: Effect of DFB on serum testosterone concentration (ng/ml) (mean ± SD). Differences were statistically
significant compared with the control value at: *** P ≤0.001

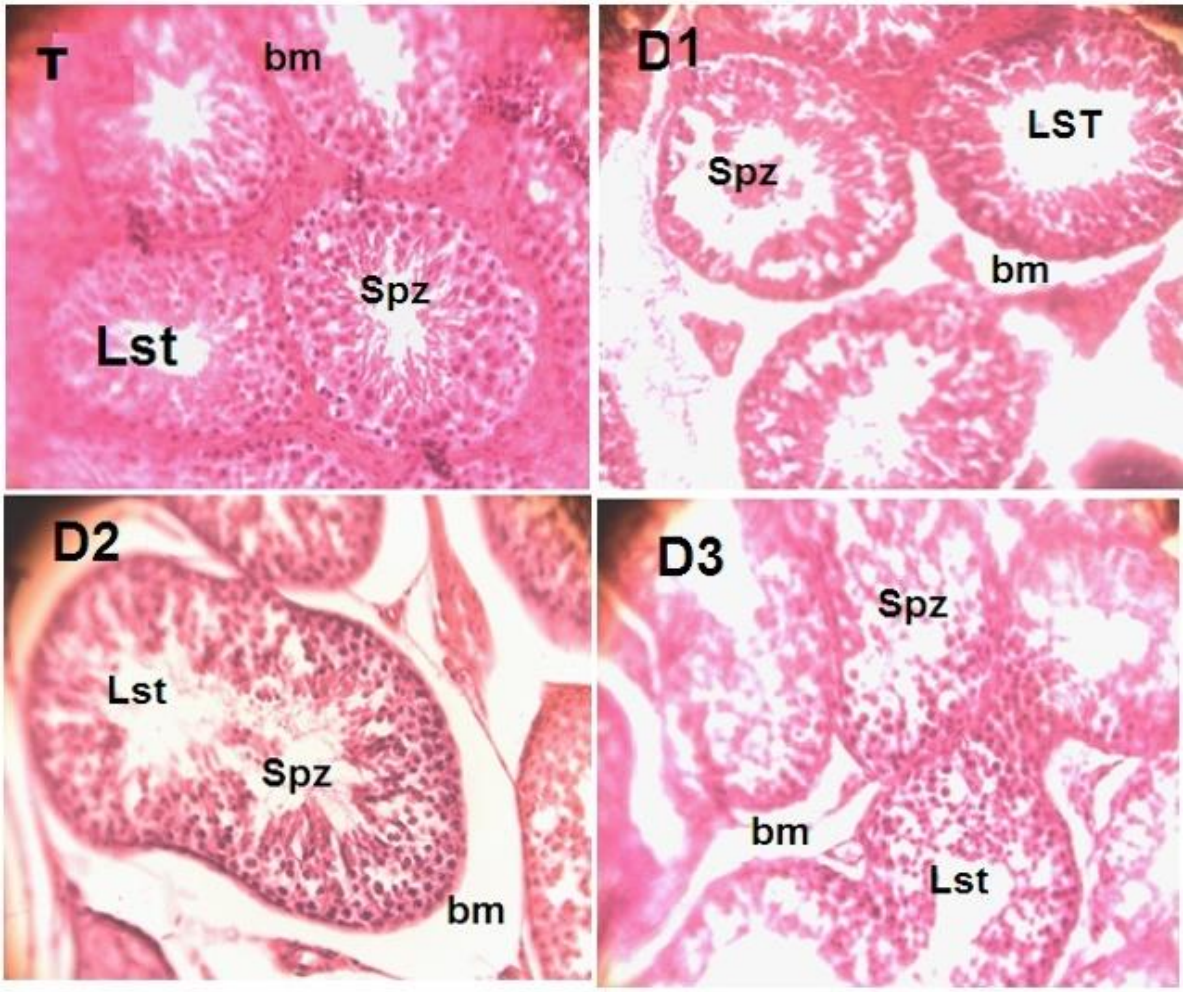


**Fig.2**: Histological Testes of rabbits from control group and treated by DFB under the microscope magnification (x 400). Spz: permatozoaLst : lumen of seminiferous tubules. bm: basal membrane.

## DISCUSSION

DFB toxicity was evidenced signed decrease in teste levels, with it's a hormone factor kay in sperm atrogenesis (Elbetieha*et al*,.2001). Numerous Factors Contribute to the reduction of Test by inhibiting steroid, a defect in the Product of androgers, or degenerat of leydig the level of the test is reduced (Taib*et al*,.2014), achange in the level of test, LH and FSH Can disrupt the Process of sperms production (Faraga*et al*,.2010). In addition pesticides cause increase secretion of the hypothalamic corticotropin-releasing hormone with stimulates the release of adrenocoticotropic hormone (ACTH) and cortisol (Larsen *et al*,.1998) ,high cortisol level inhibit testosterone level. For the production, maturation,and transfer of sperms, the existence of appropriate levels of sex hormone is essential ,a change in the level of test .L,H, and FSH can distrupt the process of sperm product (Frang *et al*,.2010) some studieswereconfirmed our work by (Yousef *et al*,.2003) who reported impairments of cypermethrin on mal rat reproduction system similar effects were observed by (Issam*et al*,.2011) after subcutaneous PM treatment.

Exposure to pesticides interferes with spermatogenesis by damaging the testes the severity of effectiondepends on the stage of differentiation and maybe lead to decrease fertility by modifying sperm characterising. (Bretveld*et al*,,2007) .

Semen quality refers to some characteristics measured in freshejaculate, the volume of the semen (mc), sperm concentration,percentage of mobile sperms, and sperms with normal.In this work, we can explainthe very high decrease of sperms in thegroup treated with DFB compared to the control group. That pesticide can affect sperms concentration by damaging the Seminiferous epithelium.through cell proliferation. (Perry *et al*,,2011). However, in high volume sperm concentration may be considerably reduced (Olivia *et al*.,2001, Yugra*et al*., 2006) suggested that pesticides exposure affects sex accessory gland: Seminal vesicles and prostate contribute to the production of 60% and 30% of the Seminal volume this may also reduce the seminal volume. Many studies have reported similar findings reduction sperm motility is correlated with the integrity of the midpiece and tail for producing energy to move. Any factor interfering with the assembling of the tail structure protein component and, ormodifying the concentration function of ATP synthesis can lead to decrease sperm motility (Perry *et al*.,2011). Our results were confirmed by (Najai*et al*, 2013) They reported that atiugine in high doses can decrease the number and progressive motility of sperms,similar results were finding by (Xia Gong et al,.2015 )Similar results were finding by (Xia *et al*.,2015) after treated a number adult rat using malathion pesticide. It was due to the decreased sperms number and motility. Histopathological examination of rabbits testes showed fission in seminiferous tubules, separation of germ cells from the base membrane, and abnormal intracellular space between seminiferous tubules (the finding were agreement with those detected by ( Heba *et al*, 2016) reported that permethrin treatment in male rats induced testicular toxicity by the biochemical and histopathological changes,same results were confirmed by (Jin *et al*.,2012) which pesticide widened interstitial space of testicular cells.In conclusion,our results revealed that diflubenzuron(DFB) caused a decrease in sperms quality and consequently influence male rabbit reproduction, studies reported that pesticides have an effect on sexual glands and reproductive system .

**Compliance with Ethical Standards:**

**Funding**: University of Badji Mokhtar Annaba-Algeria

**Animal studies**: My studies about effect of Diflubenzuron on Male Fertility in Domestic Rabbit (*Oryctolagus cuniculus*).

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