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EFFECT OF ACARICIDE TREATMENT ON BODY WEIGHT AND REPRODUCTIVE CHARACTERISTICS OF DRONES OF THE PRIOKSKY BREED TYPE OF CENTRAL RUSSIAN HONEYBEES (*Apis mellifera* Linnaeus, 1758)

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Abstract

In modern beekeeping, there is a mass death of bee colonies, one of the causes of which is varroosis. To reduce the negative impact of varroosis on the life of a bee colony, acaricides are widely used. However, the acaricidal preparations negatively affect the reproductive performance of drones. Various reports note their ambiguous effect on some the development of individuals in a bee family, including drones, and, consequently, economically useful traits (honey and wax productivity, queen egg production, resistance to diseases). In this work, for the first time, we obtained data that acaricidal preparations of amitraz, fluvalinate and thymol + oxalic acid negatively affect the reproductive performance of honeybee drones of the Central Russian breed and cause a deterioration in sperm quality. The aim of the work was to study the effect of acaricidal preparations on the fertility, deformation and concentration of spermatozoa of honey bee drones of the Prioksky breed type of the Central Russian breed, as well as on their body weight. The work was carried out at the experimental apiary of the Federal Beekeeping Research Centre (Rybnoye, Ryazan Province, spring-summer 2021). Colonies of Central Russian bees (*Apis mellifera* Linnaeus, 1758) of the Prioksky breed type were assigned to four groups, three bee colonies each: group I was not subjected to treatments (control), groups II-IV were treated. The degree of *Varroa destructor* infestation was 1-2 % in all groups. After the appearance of one-day-old drone brood, each test group was treated with one of three acaricidal drugs to combat varroosis. An amitraz-based drug («Sichuan Wangshi Animal Health Co., Ltd.», China; hazard class 3, SanPiN 1.2.25.84-10) was used in group II, a fluvalinate-based drug («Shanxi Zhenxing Fish & Bees Medicine Industry Co., Ltd.», China; hazard class 3, SanPin 1.2.25.84-10) in group III, and a drug containing oxalic acid («Shandong Deshang Chemical Co., Ltd.», China) and thymol («Hunan Insen Biotech Co., Ltd.», China) (hazard class 4, SanPin 1.2.25.84-10) in group IV. Preparations containing the active ingredients amitraz and thymol + oxalic acid were sprayed onto drone brood in 2 repetitions with 7-day interval. The fluvalinate-containing strips were placed on both sides of the drone brood frame. The impact of antivarroal drugs was assessed based on sperm quality parameters and body weight of drones at the age of 26-30 days. Sperm was collected by artificially stimulating endophallus eversion in mature drones aged 26-30 days. The concentration of spermatozoa, motility, viability, morphology (defects and abnormalities) were assessed. When assessing the viability of spermatozoa by fluorescent microscopy, fluorochromes Hoechst 33258 (Pan-Eco, Russia) and PI (Khimmed, Russia) were used with a biological luminescent light-emitting diode microscope MICROMED 3LYUM LED (OOO Observational Instruments, Russia) with 400× magnification. To determine the deformation of the heads of spermatozoa, rapid differentiated staining with a set of reagents Diahim-Diff-Quick (OOO "ABRIS + NPF", Russia) was used. Our results show that the treatment of bee colonies with acaricides affects the weight of drones. The decrease in body weight was significant when using fluvalinate and oxalic acids + thymol preparations, where the maximum weight of drones was 10-20 mg less ($p < 0.05$). It was found that sperm quality parameters decrease after treatment with acaricides. The viability of spermatozoa decreased by 1.3 % on average, sperm concentration decreased 2.2 times, and the number of spermatozoa with abnormal head morphology increased 1.3 times. Therefore, acaricides

should be used only for medicinal purposes when varroasis is confirmed. The frequency of use should depend on the degree of invasion in order to reduce the negative impact of drugs on the reproductive function of drones.

Keywords: *Apis mellifera*, honey bee, drone, sperm quality, spermatozoa viability, spermatozoa morphology, acaricidal preparations, amitraz, fluvalinate, thymol, oxalic acid

Beekeeping is an important element of the agro-industrial complex. Honey bees as pollinators account for about 80% of entomophilous plants. Bee breeding is important for increasing the biodiversity of pollinators in the ecosystem, increasing the yield of entomophilous agricultural crops, obtaining dietary foods, medicinal preparations for apitherapy, and various raw materials for processing [1, 2]. In apimonitoring, on the basis of which the state of environmental pollution is assessed and monitored, honey bees and their products are used as a bioindicator [3, 4].

In Russia, as well as throughout the world, there is a mass death of bee colonies, known as colony collapse disorder (CCD) [5-7]. The causes of CCD are massive use of pesticides, including neonicotinoids; uncontrolled breeding of bees, which leads to mass hybridization; varroosis caused by the ectoparasite *Varroa destructor* which also is a carrier of viral diseases [8, 9].

A serious factor damaging beekeeping is the massive use of pesticides, which causes the death of bee colonies, and residues of harmful substances are found in hives, in beekeeping products, as well as in adult bees and bee brood [10, 11]. The accumulation of pesticide residues in the nests of bee colonies can lead to deterioration in their health and development [12]. Honey bees exposed to pesticides become susceptible to infection by the microsporidia *Nosema ceranae* and other diseases [13, 14]. Treatment of bee colonies infected with *Nosema ceranae* with fipronil has been shown to have a negative effect on drone fertility [15].

Varroosis is widespread throughout the world. The consequences of a high degree of invasion in a bee colony may be a reduction in the number of drones, a delay in their development, and a decrease in weight. With a high degree of invasion, drones develop pathologies in the form of underdeveloped wings or their complete absence, and life expectancy is reduced, i.e., the most drones do not survive to sexual maturity [16]. The main method of combating and preventing varicose veins is the use of organic substances of natural origin (formic acid, oxalic acid, thymol), synthetic compounds (acaricides based on amitraz, fluvalinate, coumaphos, etc.), as well as raw materials of medicinal plants [17, 18].

It has been established that in the case of high infestation, when families are treated with a drug containing the active ingredient fluvalinate (concentration 10%, in strips), drones are significantly reduced in number [19-21]. Also shown is high mortality when treated with drugs containing fluvalinate (20.4 mg/100 ml of acetone) in drones aged 12 to 18 days (66.9% death). In surviving drones after treatment with this drug, a decrease in body weight of approximately 5-10% and in the length and width of the right forewing occurs [21]. It has been established that the sperm concentration in drones treated with drugs containing the active ingredients fluvalinate and amitraz decreases compared to untreated drones [22].

Bees treated with 30% formic acid remove drone brood, development of drones is delayed and their survival rate is reduced, but the organic acid does not have a negative effect on the mass of seminal vesicles and mucous glands. It has been hypothesized that thymol treatment may reduce drone flight activity [23].

The use of coumaphos has a negative effect on bee colonies: high concentrations impair memory, affect movement, as well as the behavior of nurse bees, and reduce trophallaxis of honey bees. Coumaphos (2-5 μg) has also been shown to negatively affect uterine development, including leading to a decrease in body weight [24-26]. Coumaphos caused a decrease in sperm viability and concentration

in semen immediately after collection, as well as in samples stored for up to 6 weeks [21]. A decrease in the mitochondrial activity of sperm in drones and, accordingly, their viability under the influence of imidacloprid (200 μg) has been detected [27]. Sublethal doses of fipronil during puberty resulted in decreased sperm concentration, lower sperm viability, while increased metabolic rate [28, 29].

The pesticides listed above are most effective in combating the *Varroa destructo*r mite, but they all have a negative impact on the life of the bee colony. Any such effects of treatment with one of the acaricides may affect the reproductive function of the queen and drones. Exposure to acaricides during drone development reduces drone viability and body weight, including reproductive gland weight and sperm concentration [30]. It is important to note that drones, by fertilizing the queen bee, take part in the transfer of genetic material to the next generation. A decrease in sperm viability due to treatment with anti-borroosis drugs can negatively affect the overall development of the bee colony.

Currently, there is insufficient data on the effect of acaricides on the physiological state of bees, and especially queen bees and drones, including their reproductive functions [31].

In the presented work, we for the first time obtained data that acaricidal preparations, which include amitraz, fluvalinate and thymol with oxalic acid, negatively affect the reproductive performance of drones of honey bees of the Central Russian breed and cause a deterioration in the quality of sperm.

The purpose of the work was to study the effect of acaricidal drugs on fertility, deformation and concentration of spermatozoa of drones of honey bees of the Prioksky breed type of the Central Russian breed, as well as on their body weight.

Materisl and methods. The work was carried out at the experimental apiary of the Federal Scientific Center for Beekeeping (Ryazan Province, Rybnoye) in the spring-summer period of 2021. From bees (*Apis mellifera* Linnaeus, 1758) of the Prioksky type of the Central Russian breed, 4 groups of bee families were formed, identical in economically useful traits, group I for control (not subjected to treatment), group II-IV for test treatments. Each group had three bee families (8 bee colonies, 3-5 frames with brood, 10 kg of honey, 2 kg of bee bread per family). The infestation by the *Varroa destructor* mite was 1-2%. After the appearance of 1-day-old drone brood, each experimental group was treated with one of three acaricidal drugs to combat varroosis, in group II, with an amitraz-based drug (hazard class 3, SanPin 1.2.25.84-10; Sichuan Wangshi Animal Health Co., Ltd., China); in group III, wirh a fluvalinate drug (hazard class 3,, SanPin 1.2 .25.84-10; Shanxi Zhenxing Fish & Bees Medicine Industry Co., Ltd., China); in group IV with a drug with oxalic acid (Shandong Deshang Chemical Co., Ltd., China) and thymol (Hunan Insen Biotech Co., Ltd., China) (hazard class 4, SanPin 1.2.25.84-10).

Preparations containing the active ingredients amitraz and thymol with oxalic acid were sprayed onto the drone brood in duplicate after 7 days. Strips with the fluvalinate-based drug were placed on both sides of the drone brood frame. In all groups, frames with drone brood were placed in isolators 2-3 days before the drones emerged; after the drones emerged, each was marked with permanent markers of different colors depending on the group.

The body weight and sperm quality indicators of drones were determined at the age of 26-30 days. To determine the mass of drones, laboratory analytical balances AND GR-200 (A&D Co., Ltd., Japan) were used. The measurements were carried out in triplicate, $n = 30$ from each test group. Sperm was collected by artificially stimulating endophallus eversion from sexually mature drones aged 26-30 days.

The concentration [32] and motility of spermatozoa [33] were determined using a Goryaev camera (MiniMed, Russia), viability was determined by membrane integrity [34] using fluorescence microscopy, and the morphology of spermatozoa (defects, anomalies) was also examined [35]. To assess sperm viability, fluorochrome Hoechst 33258 (Pan-Eco, Russia) and PI (Khimmed, Russia) were used. Working solutions of fluorochromes were prepared in Tris buffer (pH 8.8); the final concentration of Hoechst 33258 is 5 μg/ml, of PI is 10 μg/ml. A suspension from the sperm sample was prepared in Tris buffer (pH 8.8) at a ratio of 1:400. The studies were carried out on a biological luminescent LED microscope MICROMED 3 LUME LED (Observational Instruments LLC, Russia) at a magnification of ×400. A total of 200 spermatozoa were counted. To determine the deformation of sperm heads, we used the rapid differentiated staining with Diachim-Diff-Quick reagents (NPF ABRIS+ LLC, Russia).

The results obtained were processed using Statistica software for Windows version 13 (StatSoft Russia, Russia) and Microsoft Excel 2010 by common methods of variation statistics and assessment of the significance of differences by the Student's *t*-test. For each series of data, the arithmetic mean (*M*), standard deviation (±SD) and coefficients of variation (*Cv*) were calculated. Differences between indicators were considered statistically significant at $p < 0.05$.

Results. In honey bees, body weight is an important physiological indicator on which many characteristics depend, including the ability to overwinter for a long time and the maximum supply of nectar to the nest. In a queen bee, body weight characterizes her ability to produce high eggs; in drones, it is an indicator of viability and fertilizing ability.

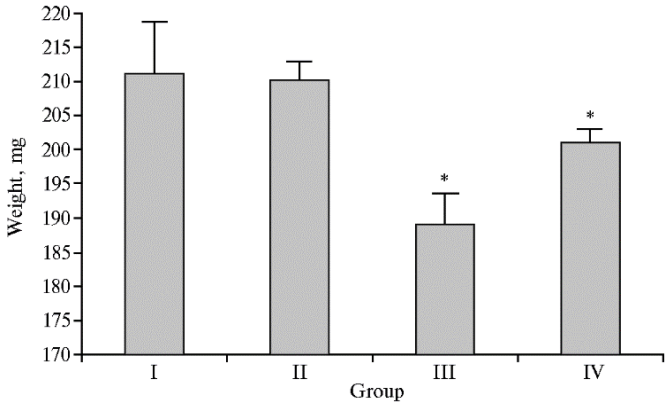

Fig. 1. Body weight of the Central Russian breed (Prioksky type) drones (*Apis mellifera* Linnaeus, 1758) after drone brood treatment with acaricidal preparations ($N = 3$, $n = 30$, M±SD; Rybnoye, Ryazan Province, 2021). For a description of the groups, see the Materials and methods section.
* Differences from control are statistically significant at $p < 0.05$.

In the studied groups, the body weight of drones varied significantly (Fig. 1). In the test groups it was 1-22 mg less than in the control. Statistically significant differences of 5-10% ($p < 0.05$) were established for drones from groups III and IV. Therefore, the negative effects of amitraz on the weight of drones, in contrast to fluvalinate and oxalic acid with thymol, was not observed.

To obtain freshly selected sperm and determine its quality indicators, 100 drones were selected into cages and the ratio of sexually mature drones to their total number was determined (Fig. 2). The largest proportion of sexually mature drones was in the control group. In the experimental groups this indicator decreased. The most noticeable decrease occurred in group III, by 10% vs. control.

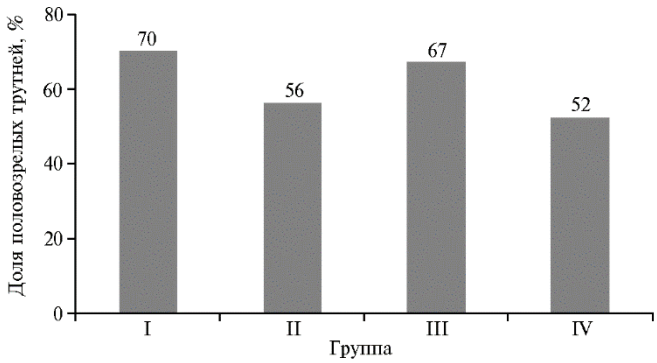

Fig. 2. Percentage of mature Central Russian breed (Prioksky type) drones (*Apis mellifera* Linnaeus, 1758) from the total number of drones after drone brood treatment with acaricidal preparations (n = 100; Rybnoye, Ryazan Province, 2021). For a description of the groups, see the Materials and methods section.

Quality parameters of freshly collected sperm of Central Russian breed (Prioksky type) drones (*Apis mellifera* Linnaeus, 1758) after drone brood treatment with acaricidal preparations (N = 3, n = 200, M±SD; Rybnoye, Ryazan Province, 2021)

Parameter		Group			
		I (control)	II	III	IV
Viability, %	M±SD	99.1±0.52	97.9±0.94	99.4±0.19	97.6±0.48
	Lim	98.3-100.0	96.8-99.7	99.2-99.7	96.7-98.2
	Cv, %	0.9	1.6	0.3	0.8
Deformation of sperm heads, %	M±SD	52.0±0.63	72.9±2.86	52.3±12.64	68.7±0.88
	Lim	51.0-53.1	69.5-78.7	28.8-71.7	67.1-70.2
	Cv, %	2.0	6.8	41.6	2.4
	td		7.06*	0.02	15.5*
Concentration, $\times 10^6$/μl	M±SD	1.9±0.12	1.1±0.21	1.8±0.63	0.7±0.05
	Lim	1.7-2.1	0.7-1.5	0.6-2.8	0.6-0.8
	Cv, %	11.2	38.7	61.7	13.6
	td		3.7*	0.2	9.2*

N o t e. For a description of the groups, see the Materials and methods section.
* The changes vs. control are statistically significant at $p < 0.05$.

The qualitative indicators of sperm, which determine its fertilizing ability, change depending on external factors. Important criteria in assessing the quality of drone sperm are viability, concentration, and the morphological structure of the sperm head (Table).

Sperm viability in drones did not have statistically significant differences vs. control. A slight decrease in sperm viability, by 1.2-1.5%, was detected in groups II and IV where minimal values of this indicator were also noted.

In groups II and IV, we found a statistically significant increase in the proportion of damaged sperm with deformed heads. By 1.4-1.3 times ($p < 0.05$) compared to the control group. It should be noted that the minimum number of damaged sperm in drone sperm was recorded in group I (control), which is significantly ($p < 0.05$) less than in the experimental groups, and is confirmed by the low value of the trait variability coefficient.

With rapid differential staining with a set of Diachim-Diff-Quick reagents, we discovered various anomalies of sperm heads in drones (Fig. 3).

In group II and group IV, the sperm concentration statistically significant ($p < 0.05$) decreased 1.8-fold and 2.7-fold vs. control, respectively, therefore, this significantly reduced sperm quality. It should be noted that lower sperm concentrations were noted in all experimental groups and, compared with group I, this indicator was on average 2.7 times lower, which confirms the negative impact of acaricides on the quality indicator of drone sperm.

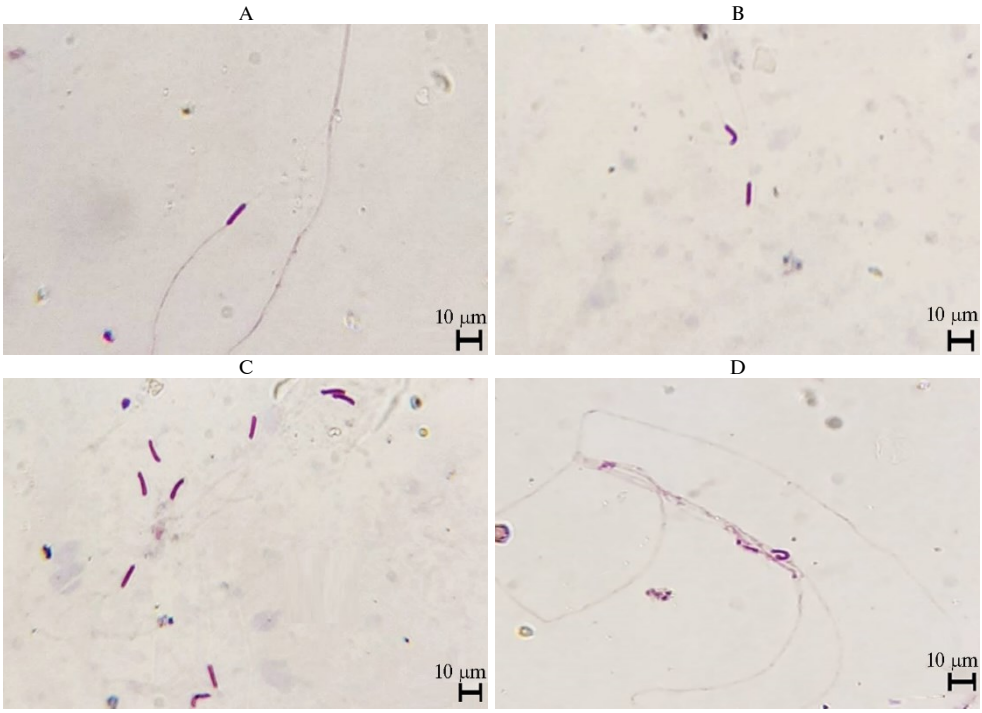

Fig. 3. Deformed heads of spermatozoa in the Central Russian breed (Prioksky type) drones (*Apis mellifera* Linnaeus, 1758) after drone brood treatment with acaricidal preparations: A — normal sperm head without deformation (control), B, C — pathologies of sperm heads in group II, D — deformations of sperm heads in group III (rapid differential staining with a set of reagents Diahim-Diff-Quick, OOO NPF ABRIS+, Russia; microscope MICROMED 3 LUME LED, OOO Observational devices, Russia, magnification ×400; Rybnoye, Ryazan Province, 2021). For a description of the groups, see the Materials and methods section.

Until now, the effect of anti-varroa drugs on honey bee drones has not been fully studied. Although detailed preparations have found fairly widespread use among beekeepers, information about their effect on the quality and reproductive properties of drones is extremely limited [31]. In the experiments of F.B. Abdelkader et al. [36] it was found that in the case of the drug with the active substance amitraz Rulamit-VA (TEKNOVET İLAÇ SANAYİ VE TİCARET ANONİM ŞİRKETİ, Turkey), used according to the manufacturer's instructions, high spermatozoa mortality and a high percentage of spermatozoa with impaired membrane integrity and acrosome defects were detected. Treatment with oxalic acid (spraying 5 ml per frame space) led to a decrease in the concentration and motility of spermatozoa and to violation of the acrosome integrity. A review article by J. Rangel and A. Fisher [30] provides information on the effect of acaricides on the parameters of drone sperm. Thus, in drones from bee colonies exposed to fluvalinate (20.4 mg/100 ml acetone), thymol (at concentrations below LD_{10}, the norm for worker bees) and amitraz (4.3 mg/100 ml acetone), the sperm concentration was lower than in other experimental groups and in the control. Drone mortality was higher in fluvalinate-treated colonies (66.9%) compared to untreated colonies (62.5%).

In our work, we also monitored the effect of acaricidal drugs on the fertility of drones, however, unlike foreign researchers, we sprayed 1-day-old drone larvae and evaluated the effect of the drugs at the larval stage of development.

Thus, the negative impact of acaricidal drugs, which include amitraz, fluvalinate and thymol with oxalic acid, on the development of drones of honey bees of the Central Russian breed and the quality of their sperm has been shown. The

body weight of drones significantly decreased, by 10-20 mg, afther treatment with fluvalinate and oxalic acid + thymol. Acaricides decrease sperm viability on average by 1.3%, sperm concentrations decreases 2.2-fold and the number of spermatozoa with deformed heads in the spermatheca is 1.3 times higher. All tested substances had an equally negative effect on drones. Therefore, acaricides should be used only for medicinal purposes when mite infestation is confirmed. The frequency of tretments should depend on the degree of invasion. To reduce the negative impact of drugs on the reproductive function of drones, do not exceed the doses indicated in the instructions.

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