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Original Article

Control of *Varroa Destructor* in Kazakhstan

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Abstract

Varroa mite is one of the most dangerous bee parasites and causes a lot of damage to the beekeeping industry. Several chemical and herbal medicines have been used to control this mite so far. In this study, the effects of *Artemisia absinthium* and *Hypericum perforatum* prescription to control *Varroa* mite were investigated. To this end, a total of 380 bee colonies in 5 areas in Kazakhstan were considered. The bitter wormwood herbs and *Hypericum* extracts were mixed in a ratio of 1:1, and then, prepared in a ratio of 1:10 with sugar syrup (100 ml infusion per 1 liter of sugar syrup). An amount of 200 ml of the prepared solution was used for 5-7 days per colony after medical collection and honey pumping. The percentage of mite infestation at the beginning and end of the experimental period, which was spring, summer, and autumn, and the number of worker mites and worker bees were determined daily. At the beginning of the experiment, the percentage of mite infestation in the experimental hives was not significantly different; however, at the end of the experiment, there was a significant difference between the experimental treatments. The use of a mixture of *A. absinthium* and *H. perforatum* extract significantly reduced the abundance index of *Varroa* mite to 0. Due to the adverse effects of chemical drugs and their high cost, it is recommended to use this extract to control *Varroa* mite contamination.

Keywords: *Apis mellifera* L.,*Artemisia absinthium, Hypericum perforatum,* abundance index,*Varroajacobsoni*

1. Introduction

The bee, like other living organisms, has been exposed to numerous pests and diseases during its lifetime, and one of the most common and dangerous pests that severely affect the population and performance of the bee colonies is the *Varroa* mite. This mite belongs to the Varroidae family and *Varroa* genus and feeds on adult larvae hemolymph, pupae, and bees at all stages of life. Mite colony infection causes the bees to lose weight, deform, or lose limbs, and sometimes make young bees die. *Varroa* mite is one of the most important and destructive pests of beehives that causes irreparable damage to the beekeeping industry (1-3). Numerous researchers have tried to find ways to control the parasite, and in this regard, such chemicals as Bayvarol, Apistan, Apigard, and Folbex have been reported to be able to prevent the outbreak of *Varroa* mite to some extent (4-6).

The *Varroa* mite is undoubtedly the most important pest and a serious threat to the beekeeping industry worldwide in the 21st century. Although some colonies are less affected, some colonies die within a few years despite a strong population. At present, there are negative trends in beekeeping in Kazakhstan, as a result of both socio-economic processes and the deterioration of the epidemiological and environmental situation. Brood diseases, such as Varroatosis, Ascospherosis, and Nosemosis, are widely distributed in Kazakhstan. *Varroa destructor* is the mite responsible for varroatosis (or varroosis), an external parasitic disease that attacks honeybee colonies (adult bees, especially the brood), which causes major economic losses to the

beekeeping sector (7). *Varroa*mite can transmitpathogenic viruses to honey bees, often associated with colony collapse (8-10), and cause negative effects on the bee's immune responses (11-13).

There are numerous methods, including physical, biological, and chemical, which are used to control the *Varroadestructor*. Chemical pesticides (synthetic varroacides) are often employed to reduce the *Varroadestructor* population. Pyrethroids, such as *Tau*-Fluvalinate and Formamidine Amitraz are used for *Varroa* control in Kazakhstan (14, 15). However, the use of chemical pesticides reveals numerous problems. This is the contamination of bee keeping products with their residues and metabolites (16, 17), toxic and side effects of drugs on bees (18), also *Varroa* mites develop resistance to chemicals (19, 20).

Today, various efforts are being exerted to replace herbal medicines with chemical ones in different countries (21-24). Among the medicinal plants that have been used in the control of *Varroa* mites by various researchers, including pepper, mint, chicory, and lavender (21, 22).The two medicinal plants, namely *Artemisia absinthium* and *Hypericum perforatum*, have also been used for a long time due to their numerous properties. *Artemisia absinthium,* which is called with numerous synonymous Latin names, contains various compounds responsible for its biological activities. The most common compounds are thujyl alcohol esters, *α*-thujone, *β*-thujone, camphene, *α*-cadinene, guaiazulene (*Z*)-epoxyocimene, (*E*)-sabinyl acetate, (*Z*)-chrysantenyl acetate (25). *Hypericum perforatum* is a perennial flowering herb belonging to the Clusiaceae family, consists of approximately 500 species, and has been considered a medicinally valuable plant for over 2,000 years. The most important metabolites present in *H. perforatum* are phloroglucinols (hyperforin, hyperforin), naphtodianthrones (hypericin, pseudohypericin), flavonoids (rutin, quercetin, quercitrin, isoquercitrin, hyperoside, and amentoflavone), phenolic acids, and small amounts of essential oil (26, 27).

The control of *Varroa jacobsoni*over *Apis mellifera* L. was easily achieved during the 1980s with the production of effective acaricides (*Varroadestructor*), and it appeared that the *Varroa* mite problem was solvable; however, experimental results showed that the mite-resistant line caches have been created. Resistance of *Varroa* mite to fluovinate acetate (an effective Pyrethroid acaricide against *Varroa* mite, of which Apistan is a well-known example) was reported first in Italy and then in other countries. Gradually, it became clear that only a small number of chemical acaricides were suitable for controlling mites. Fumisan also comes in the form of wood strips. Their width, length, and thickness are25 mm, 2 cm, and 1 mm, which come in 10 pcs in a package. They are infused with acaricide, a substance that kills ticks. The active ingredient in Fumisana is fluvalinate.

The effectiveness of miticides is greatly influenced by the physiological condition of bee families, weather, and natural climatic conditions. In this regard, it is necessary to study the involvement of bees with varroatosis and the effectiveness of miticides used to control bees in the conditions of Kazakhstan. This study aimed to evaluate the different acaricides regarding the duration (days) of effectivenessand compare with control (i.e., untreated group of honey bee colonies).

2. Materials and Methods

2.1. Site Location

This study was conducted at the laboratory of Anti-Parasitic Biotechnology, Department of Biological Safety, Kazakh National Agrarian University, Kazakhstan, and on the apiary of the Almaty, Turkestan, and East Kazakhstan regionsfrom 2018 to 2021 (Figure 1). In this experiment, raw data were obtained from diagnostic and experimental therapies. A total of 380 bee colonies from the native population were used to determine the effectiveness of the recommended drugs for the control of *Varroa* mites. Studies were carried out both on barren bee families

and families with a spread in the summer and autumn periods.

Figure 1. Site locations
[1]Karasai district, [2]Enbekshikazakh district, [3]Talgar district, [4]Tole bi district, [5]Altai district

2.2. Treatment Preparation

In this experiment, the treatment methods included infusion of herbal solution from bitter wormwood herbs (*A.absinthium)* and *H. perforatum*. The bitter wormwood herbsand *Hypericum*extracts were mixed in a ratio of 1:1, and then, prepared in a ratio of 1:10 with sugar syrup (100 ml infusion per 1 liter of sugar syrup). An amount of 200 ml of the prepared solution was used for 5-7 days per colony after medical collection and honey pumping.This experiment was performed using "Methodological recommendations for the study of drugs and methods for combating varroatosis of bees" (28) and methodological guidelines for the production of experiments in beekeeping (29, 30).

Bees were kept in 16-frame hives, and oxalic acid was applied for the treatment of bees. The gluing bees in barren families were determined by sampling bees from the center of the nest into a folded sheet of paper in the amount of 100-150 individuals, in families with a spread by a sampling of 100 doll bees in the sealed melt. The collected samples were stored in the freezer.The experimental treatments were sprayed using a syringe in the space between the bee frames (Figures 2-4).

Figure 2. Treatment of bees with a syringe

Figure 3. Bees and apiary

Figure 4. Bees affected by tick-borne Varroatosis

The presence and number of mites on the bees were determined individually or by group sampling method. In a group study, a container of honey, a net or gauze (to prevent mites to pass through), a small amount of washingpowder, and a piece of plastic or cloth were prepared for the determination of the number of bees' mites(31).Bee samples were placed in heat-resistant cups with a capacity of 300-400 ml, 3-5 g of washing powder was added, andthen, 300-400 ml of boiling water was poured and stirred periodically for 5-10 min. Afterward,a solution with the bees was poured into the device, where the mites settled on the bottom of the

funnel. Subsequently, the clamp was removed, the solution was drained into a used glass, and the number of mites was counted. Infected cells were sampled by opening the cell lid with a scalpel and examining the pupa individually. Only adult *Varroa* mites were considered. Once the parasites were identified, the data of bees or pupae were recorded with an abundance index, calculated by the number of mites (32). The obtained data were subjected to biometric analysis according to the methods proposed by Chiou (33).

2.3. Determining *Varroa*mite in Honey Bee Colonies

The degree of infection of bee colonies in *Varroa* mites was determined by the following formula:

$$N = \frac{M}{S} \times 100$$

where:

N: *Varroa*mite infestation levels in honey bee colonies (number of mites per 100 bees);

M: number of mites found

S: number of bees in the sample

The level of infestation of bee colonies in *Varroa* mites is low, medium, or high if there are up to 10, up to 20, or more than 20 mites per 100 bees, respectively.

2.4. Statistical Analyses

The data were analyzed in statistical software Minitab (version 17), and the comparisons of means were performed using the independent T-test. Excel software was used to draw graphs and tables.

3. Results

3.1. General Characteristics of Apiary

In this study, 380 bee colonies were studied located in zones Almaty, Turkestan, and East Kazakhstan regions, Kazakhstan. Table 1 showed the apiary, area/district, number of bee colonies, number of infected colonies, and percentage of infection. To pre-diagnose and determine the spread of varroatosis on the apiary farms of various regions from 2018 to 2021, studies were carried out by clinical examination of bee families.The highest levels of *Varroa* mite infection are related to Talgar district (15%) and Enbekshikazakh district (12.86%) regions, Kazakhstan, and the lowest levels of pollution are related to Tole bi district (0.00%) and Karasai district (3.33%), Kazakhstan.

Table 1. Results of the study of bee colonies for varroatosis mites

Apiaries	Region/District	Number of honey bee colonies	Infestation level of *Varroa*	
			Number of Infected bee colonies	Infestation (%)
P.P.Konovalov	Almaty region, Karasai district, Gorniysadovod	120	4	3.33
V.S Savarovsky	Almaty region, Enbekshikazakh district, Issyk city	70	9	12.86
Bisen Ata	Almaty region, Talgar district, Taldybulak village	20	3	15.00
M.Yrysbaev	Turkestan region, Tole bi district, Kazakhstan settlement	80	0	0.00
Arashy	East Kazakhstan region, Altai district, Putintsevo village	90	5	5.56
Total		380	21	5.53

3.2. Percentage of hives Infested with *Varroa* Mites

In different seasons, the percentages of infestation of experimental hives in different areas was determined at the beginning and end of the experimental period and showed that at the beginning of the experiment there was no significant difference in terms of *Varroa* mite infestation; however, at the end of the experiment, a significant difference was observed using experimental treatments (Table 2). Based on figure 5, the infestation intensities of adult and larvae bees in spring, summer, and autumn were calculated at 1.5, 1.7, and 2.1, 1.3, 1.6, and 1.9, respectively.

Table 2. Seasonal intensity of invasion of bees by varroatosis

Ticks detected	Spring (April)		Summer (June)		Autumn (October)	
	Total	II[1]	Total	II	Total	II
On bees	375.00	1.50	425.00	1.70	525.00	2.10
On larva	325.00	1.30	400.00	1.60	475.00	1.90

[1]: Intensity of infection

The infection intensity rates of adult bees'samples in spring, summer, and autumn were estimated at1.5%, 1.7%, and 2.1%, respectively. At the beginning of the queen spawning season (spring), with increasing hive activity and reproduction, the percentage of intensity of infection increased, which was also observed in bee larvae. A sampling of *Varroa* mites from the mentioned apiaries showed that they were 1.13 and 1.40 greater in summer and autumn than in spring, respectively, the intensity of infection was increased, and this trait on the larvae increased by 1.23 (summer) and 1.46 (autumn) times.

The effect of plant extract on the abundance index before and after the experiment was shown in table 3. According to different samplings, the abundance index before using experimental treatments varied from 0.01 to 0.05, which reached 0after using plant extracts. Nevertheless, in the control group, the abundance index increased, and efficiency in processing was calculated to be 100 and 0.00 in the experimental and control groups, respectively.

The effect of plant extract on the abundance index was also calculated before and after the experiment in autumn (Table 4). The results showed that the abundance index before using the experimental treatments ranged from 0.036 to 0.082, which reached 0after using plant extracts. Nonetheless, in the control group, the abundance index increased from 0.019 to 0.068, and efficiencies in processing were calculated to be 100 and 0.00 in the two experimental and control groups, respectively.

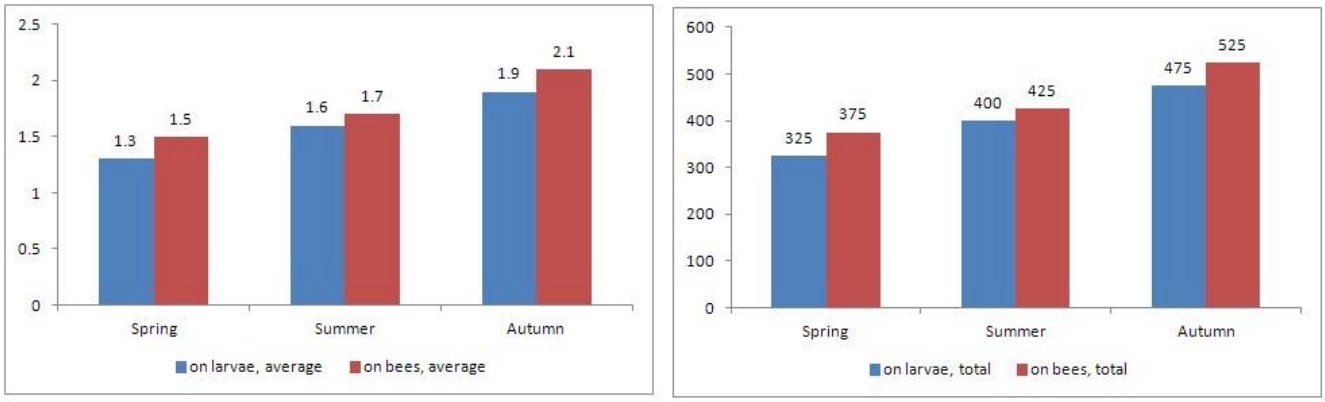

Figure 5. Seasonal infestation intensity of bees and larva by *Varroa*mite

Table 3. Botanical efficiency in the summerseason

Family group	n	Abundance index		Efficiency in processing (%)
		Before treatment	After treatment	
Experimental	45	0.02	0.00	100
	36	0.01	0.00	100
	39	0.01	0.00	100
	16	0.05	0.00	100
Control	12	0.03	0.04	0.00
	7	0.02	0.02	0.00
	3	0.03	0.04	0.00

Table 4. Botanical drug efficiency in the autumn season

Family group	n	Abundance index		Efficiency in processing (%)
		Before treatment	After treatment	
Experimental	48	0.064	0.00	100
	24	0.036	0.00	100
	25	0.082	0.00	100
Control	15	0.021	0.019	0.00
	27	0.048	0.046	0.00
	23	0.056	0.068	0.00

Currently, a large number of drugs have been proposed for the therapy of bee families infested with varroatosis. Beekeepers, when choosing drugs, often focus on information emanating from product manufacturers. However, to the best of our knowledge, there is little data on the efficacy of acaricides used to treat bees. In the conditions of the apiary farms of Talgar district, Enbekshikazakh district, Karasai district, Tolebi district, and Altai district regions, Kazakhstan, the anti-adhesive activity of botanical drug composition and Fumisan were studied in the present research (Figure 4). Table 5 showed that the use of Fumisanin both experimental and control groups was as effective as herbal medicines and reduced the abundance index in the experimental group from 0.02 to 0.07 and reached 0.00.

Table 5. Fumisan efficiency in the autumnseason

Family group	n	Abundance index		Efficiency in processing (%)
		Before treatment	After treatment	
Experimental	46	0.02	0.00	100
	4	0.07	0.00	100
	5	0.02	0.00	100
Control	8	0.01	0.02	0.00
	17	0.02	0.03	0.00
	33	0.02	0.03	0.00

4. Discussion

The results of the colonies involved in *Varroa* mites in spring, summer, and autumn indicated that after the beginning of the bee breeding season in spring, the number of mites also increased over time. In most parts of the world, including Kazakhstan, hives infected with *Varroa* mites are treated with chemical drugs. In addition to adverse effects on bee colonies, chemical drugs remain in bee products. Moreover, if such chemicals are used, drug resistance develops in bees. The results of this study showed that the use of *A. absinthium* and *H. perforatum* led to the proper control of the involved apiaries of *Varroa* mites. *α*-Thujone is commonly considered to be the principal active ingredient of *A. absinthium* and toxic principle in absinthe (34) and it was one of the two most toxic monoterpenoids tested against western corn rootworm larvae. Public suspicion of synthetic pharmaceuticals and pesticides has led to the growing popularity of herbal medicines and botanical insecticides even though they have not been subjected to the same severe tests of safety and evaluation of toxicological mechanisms (35-37).

The use of *H. perforatum* extracts also controlled the infection of apiary mites. *Hypericum perforatum* has a significant effect on reducing mite infestation in bees due to its composition of essential oils, phenols, and terpenes. Essential oils are one of the chemical groups in plant extracts that have a significant effect on

reducing bee and *Varroa* infection in bees (38). The compounds of phenols and terpenic in medicinal plant extracts also affect various diseases of bees, including antifungal activity. *Varroa* mite control strategy using medicinal plants has increased in recent years (5, 39, 40).

The plant compounds in the tobacco plant were also effective at controlling *Varroa* mites due to the active ingredients of nicotine, nicoteine, and nicotelline (23, 41, 42). *Peganum harmala* has long been used among Iranians as a disinfectant due to alkaloid compounds, such as harmine, harmalin, and harmalol (43, 44). Thyme kotschyonus has a disinfectant role due to its thymol and has been traditionally used in various apiaries (45).

As a result of this study about the effectiveness of drugs, it was established that in the conditions of the Almaty, Turkestan, and East Kazakhstan regions, long-acting preparations based on fluvalinate, Fumisan showed the high acaricidal effects. Considering that the active ingredient is 10 times lower in the Fumisan preparation than in Apistan, and the recommended treatment period in the presence of a melt is twice shorter (21-25 days), Fumisan is undoubtedly safer from the point of view of contamination of beekeeping products with residues of the active ingredient. It is impossible to ignore the significant difference in the price of these drugs (Fumisan is 10 times cheaper), which is important for the budget of beekeepers. Therefore, based on the results of the conducted studies, it can be concluded that botanical drugs should be in a ratio of 1:1 mixed with sugar syrup (1:10), which has high acaricidal efficiency in varroatosis of bees.

Authors' Contribution

Study concept and design: A. A. M.
Acquisition of data: A. A. M.
Analysis and interpretation of data: G. T.
Drafting of the manuscript: M. N.
Critical revision of the manuscript for important intellectual content: A. A. M.
Statistical analysis: A. A. M.
Administrative, technical, and material support: A. A. M.

Ethics

All the procedures were approved by the Ethics Committee at the Kazakh National Agrarian Research University, Almaty, Kazakhstan.

Conflict of Interest

The authors declare that they have no conflict of interest.

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