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THE INFLUENCE OF PULVERISED SUGAR DUSTING ON THE DEGREE OF INFESTATION OF HONEY BEE COLONIES WITH *Varroa destructor*

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The aim of this work was the investigation on the efficacy of pulverised sugar dusting on knocking-down Varroa destructor mites and the influence of the dynamics of the treatment on the degree of infestation in honey bee colonies. Two methods were deployed to measure the degree of infestation of bee colonies with V. destructor mites: the sugar shake method and the technique which involves mesh bottom boards equipped with sticky inserts. The research was carried out on 30 strong honey bee colonies in three successive years.

The results proved that dusting with pulverised sugar (particle diameter below 40 µm) influenced the fall of V. destructor in comparison with both their fall off prior to the treatment and the negative control. The most discernible effects on the mite fall and the decline in their population in the hives was obtained with dustings repeated at three-day intervals. To conclude, the dynamics of the treatment affected the degree of infestation in bee colonies. However, the efficacy of sugar dusting was significantly lower in comparison with the one of flumethrin (positive control). Thus, in spite of considerable favourable effects on the decline in the degree of infestation with mites, by no means may dusting with pulverised sugar be advised to beekeepers as the one and only means of bee protection against V. destructor.

The use of the technique which involves mesh bottom boards equipped with sticky inserts proved more efficacious and sensitive in the judgment of the degree of infestation of bee colonies in comparison with the sugar shake method, which includes dusting 300 bees with 40 g of pulverised sugar (particle diameter below 40 µm).

Key words: Apis mellifera, mesh bottom board, sugar-dusting, sugar-shake method, Varroa infestation

INTRODUCTION

Numerous substances and methods are suitable for the control of *V. destructor* in honey bee colonies, but none of them meets the criteria for use in

organic beekeeping. The application of acaricides in the control of the bee ectoparasite is accompanied by negative side effects on the bees themselves, beekeepers and/or bee product consumers. An additional problem is the resistance which often develops towards synthetic acaricides (Milani, 1999; Elzen *et al*., 1999; Mozes-Koch *et al*. 2000; Spreafico *et al*., 2001; Floris *et al*., 2001a; Milani i Della Vedova, 2002).

The aforementioned led to the use of an increasing number of acaricides with various mechanisms of action and, consequently, to considerable residua in bee products (Cabras *et al*., 1994; Bogdanov, 1998; Wallner 1999; Lodesani, 2008) and negative effects on bee colonies (Patetta and Manino, 1988, Rada *et al*., 1997; Pettis *et al*., 2004a). Fluvalinat, for example, significantly increases the mortality in bees and reduces the number of aerobic bacteria in their digestive system, whilst amitraz stimulates the proliferation of yeasts (Rada *et al*., 1997). Residua of cymiazole hydrochloride, a hydrosolubile synthetic acaricide, were found both in the bees and honey (Eyrich and Ritter, 1986, 1990; Omar and Shoriete, 1992; Cabras *et al*., 1994; Stanimirović 2005a). Other synthetic acaricides are liposoluble and thus leave residua in wax and propolis (Chauzat and Faucon, 2007; Martel *et al*., 2007). The remains of bromopropylate, coumaphos and fluvalinate penetrate from the comb to other parts of the hive (Wallner, 1999), into honey and virgin wax (Kochansky *et al*., 2001; Martel *et al*., 2007; Karazafiris *et al*., 2008). Short-lasting negative effects of lyposolubile acaricides result in deaths of the bees immediately after treatment (Marchetti *et al*., 1987; Floris *et al*., 2001b), while long-lasting effects give rise to disturbances in the development of bee colonies (de Ruijter and van den Eijnde, 1991) and decreasing viability and fertility of the queen. For instance, coumaphos, for instance, decreases the bodymass of the queen, and, in higher concentrations, is capable of arresting their development (Pettis *et al*., 2004a). Certain varroacides have been proved to be genotoxic (Nakano *et al*., 1996; Osano *et al*., 2002; Stanimirovic *et al*., 2003a,b, 2005a; Pejin *et al*. 2006).

Because of all aforementioned negative effects, the use of synthetic acaricides in organic beekeeping is forbidden; only organic acids (formic, lactic and oxalic) and aetheric oils (thymol, menthol, camphor, eucaliptol, camfen, p-cimene, eugenol, isopinocamphone and α-thujone) are to be used. Unfortunately, with the exception of formic acid, these substances are efficacious only in the absence of brood. Due to the fact that they evaporate easily, especially formic acid and thymol-derived preparations, the efficacy of approved substances highly depends on numerous factors (Calderone and Lin, 2003; Floris *et al*., 2004; Ostermann and Currie, 2004; Underwood and Currie, 2004, 2005). Besides insufficient efficacy, the use of organic acids and aetheric oils is acompanied by undesired efects on bees and brood (Rice *et al*., 2002; Floris *et al*., 2004; Satta *et al*., 2005; Underwood and Currie, 2003, 2005). Formic acid exerts a negative impact on bee colonies (Underwood and Currie, 2003, 2005; Ostermann and Currie, 2004) and enhances apoptosis (Gregorc *et al*., 2004). Oxalic acid is toxic (Higes *et al*., 1999; Nozal *et al*., 2003) and enhances apoptosis and sporadic cell death in the tissues of bee larvae (Gregorc *et al*., 2004).

Limited efficacies of biotechnical and biophysical methods in the control of *V. destructor* render them applicable only in combination with organic acids and essential oils (Rosenkranz *et al*., 2010). However, they alone are capable of deriving functional disturbances and deviations in the age structure in colonies. Microbial control of *Varroa* mites with fungal pathogens could be useful part of an integrated pest management program in the honey bee industry (Kanga *et al*., 2006; Miekle *et al*., 2007; Garcia-Fernandez *et al*., 2008). However, a medicinal preparation against *Varroa* mites based on entomopathogenic fungi which may enter commercial use is yet to be invented.

Above-mentioned data render ecological methods of control of *Varroa* inevitable. The basis of this combat lies in the stimulation of bee colonies to fight against *Varroa* on their own or in the invention of new formularies based on safe substances. In the current work pulverised sugar is chosen, a substance which respond to all the aforementioned demands (Fakhimzadeh, 2001c). The aim of the work was the investigation on the efficacy of pulverised sugar dusting on knocking-down *Varroa* mites and the influence of the dynamics of the treatment on the degree of infestation in honey bee colonies.

Application of pulverised sugar in combat against *Varroa* is based on the presumption that the particles adhere to the ticks' pads (Liu and Peng, 1990), thus disabling their fastening to any surface including the bodies of the bees (Ramirez and Malavasi, 1991; Ramirez, 1994). Consequently, the fall of ticks on the bottom board is facilitated, leading to their death of starvation (Ramirez and Malavasi, 1991; Ramirez, 1994). The advantages of the treatment with pulverised sugar are the fact that it lacks toxicity (Pettis *et al*. 2004b), is applicable throughout the year, including the period of honey harvest, its capability of stimulating the egg laying and brood nursing (Ambrose, 1992), and, what is possibly most important, the fact that it does not lead to resistance (Ramirez, 1994).

MATERIAL AND METHODS

Experimental design

The research was performed on 30 strong honey bee colonies in the Sjenica-Peshter region (from 43°0' to 43°30' North latitude and 19°45' to 20°45' East longitude) in three successive years. Each year sugar dusting was performed in the same period: from 2nd to 31st July. All the colonies were kept in the same type of hives (two-corporal LR hives). In the first year the colonies with one-year-old queens were homogenised and divided into five groups, each consisting of six colonies. Further on, three groups were treated with sugar, the first one nine times a month, i.e. at three-day intervals (E1J-9), the second four times a month, i.e. at seven-day intervals (E2J-4) and the third one two times a month, i.e. at fourt een-day intervals (E3J-2). One group remained untreated, being the negative control (K-J), whilst another was treated with flumethrin and was the positive control (K+J). The experiment was performed on the same groups in the second year, when the queens were two-year-old and the groups were labelled E1J'-9, 4 E2J'-9, E3J'-2, K-J' and K+J', as well as in the third year

when their queens were three-year-old (groups labelled E1J"-9, E2J"-4, E3J"-2, K-J", K+J").

The particles of the sugar applied in the experiment were of diameter below 40 μm, based on the findings of Fakhimzadeh (2000, 2001a,b), who proved that the best results of knock-down effect on *Varroa* mites were obtained with the particles measuring 25-40 μm. The appropriate dimensions of particles were obtained by grinding and sifting table sugar through a sieve with apertures sized approximately 40 μm (W.S. Tyler Industrial Group, USA). The sugar consisted of super fine pure white ground sucrose with particles of diameter up to 40 μm, which was confirmed by means of scanning electron microscopy.

At each treatment with sugar, the colonies were dusted with 40 g of sugar. The sugar was applied between the frames with a simple apparatus (Fakhimzadeh, 2000) without separation of the frames. Following the dusting of the first body in a hive, the hives were reassembled prior to dusting the second one.

Methods used for measuring the degree of infestation of bee colonies with V. destructor mites

The degree of infestation was measured prior to and following each treatment in order to judge the efficacy of pulverised sugar in knocking-down varroa mites and the influence of the dynamics of the treatment on the degree of infestation in honey bees. Two methods were deployed to measure the degree of infestation of honey bee colonies with *Varroa* mites, the sugar shake technique (300 bees in a jar dusted with 16 g sugar with particles lesser than 40 μm) and the one based on the presence of mesh bottom boards equipped with sticky inserts (Goodwin and Van Eaton, 2001; Calderone and Lin, 2003).

Statistical analysis

The experimental results were processed with statistics software GraphPad Prism 7.0. The following descriptive parameters were calculated: the arithmetic mean ($\bar{X}$), standard deviation (SD), standard error of the mean (SE), interval of variation (Min-Max) and coefficient of variation (Cv). To evaluate the significance of differences between experimental groups ANOVA was performed followed by Tukey's test at significance levels 0.05 and 0.01.

RESULTS

1. Varroa infestation levels determined by means of sugar shake method

The results of the research on the efficacy of pulverised sugar on knocking down varroas and the influence of the dynamics of the treatment on the degree of infestation of honey bees with *V. destructor* gained by means of sugar shake method are displayed in Table 1 and Figure 1.

The results showed that in the first year the experimental groups were homogenous before the treatment, since the coefficient of variation (Cv) was below 33%, that is, ranged from 11.23 to 14.00. In addition, the level of infestation

in all honey bee colonies investigated was roughly similar, ranging from 11.67 to 13.67 fallen mites on average (Table 1). The results of ANOVA showed that there were no significant differences in infestation levels between experimental groups and controls (Table 1). These results were a prerequisite for the meaningful comparison of the number of fallen mites before and after the treatment, both between the experimental groups and the control.

It is noticeable (Table 1) that in the first year the experimental groups remained homogenous (Cv<33%) after the treatment. Pulverised sugar had detectable effect on mite fall in comparison to the one before the treatment. The best effect on mite fall and consequent decline in the ectoparasite population in the hives was obtained after treatment at three-day intervals, which resulted from the decline in the average number of fallen mites in group E1J-9 from 13.33, prior to the treatment, to 10.50 after the treatment. ANOVA revealed significant ($p<0.001$) group differences in levels of Varroa infestation (Table 1). In all the groups treated with sugar there was a decrease in the degree of infestation, although it was most pronounced in the positive control (Table 1, Figure 1). According to the results of Tukey's-test, level of *Varroa* infestation significantly ($p<0.001$) differed between the positive control and each treatment group. In comparison to the negative control (K-J), group E1J-9 differed significantly ($p<0.001$), followed by groups E2J-4 and E3J-2 ($p<0.01$).

In the second year in all experimental groups the homogeneity before the treatment was existent, the Cv being well below 33%. However, significant differences ($p<0.001$) in levels of Varroa infestation between the groups (Table 1) were indicated. Tukey's test confirmed significant differences ($p<0.001$) between each treatment group (E1J'-9, E2J'-4 and E3J'-2) and the positive control (K+J'), as well as between the positive and the negative control ($p<0.001$). However, treatment groups did not differ significantly (Table 1).

In the second year all experimental groups were homogenous following the treatment (Cv<33%). ANOVA revealed significant ($p<0.001$) group differences in levels of *Varroa* infestation (Table 1). Tukey's test showed that the infestation level was significantly ($p<0.001$) lower in the positive control (K+J') in comparison with each treatment group (E1J'-9, E2J'-4 and E3J'-2). In addition, significant ($p<0.001$) differences were affirmed in comparisons of each treatment group with the negative control (K-J'). Between E1J'-9 and E2J'-4, as well as between E1J'-9 and E2J'-2 groups treated, Tukey's test revealed significant ($p<0.01$) differences, but no differences between treatment groups E2J'-4 and E2J'-2 (Table 1).

In the third year the homogeneity of experimental groups before the treatment (Cv<33%) was still present. The highest number of spontaneously fallen mites was in the negative control (34.83), and the lowest in the positive (7.67). Intergroup variations in levels of *Varroa* infestation were significant ($p<0.001$) (Table 1). Tukey's test showed that the infestation level was significantly ($p<0.001$) lower in the positive control (K+J") in comparison with treatment groups E1J"-9, E2J"-4 and E3J"-2, whilst in comparison with the negative control (K-J"), treatment groups E1J"-9 and E2J"-4 differed significantly ($p<0.001$). Group E3J"-2 did not differ significantly in comparison with the negative control (K-J"), but did significantly ($p<0.01$) in comparison with E2J"-4 (Table 1).

Table 1. Descriptive and comparative statistical analysis of levels of infestation of bee colonies with *Varroa destructor* assessed by sugar shake method prior to and following the treatment in the three-year period

Period and year of assessment	Treated group	$\bar{X}$	SD	SE	Min	Max	CV (%)
Infestation prior to the treatment in the first year	E1J-9	13.33	1.75	0.71	11.00	16.00	13.13
	E2J-4	13.50	1.52	0.62	12.00	16.00	11.23
	E3J-2	13.67	2.73	1.12	10.00	16.00	19.99
	K-J	12.17	1.47	0.60	10.00	14.00	12.10
	K+J	11.67	1.63	0.67	10.00	14.00	14.00
Infestation following the treatment in the first year	E1J-9	10.50yw	2.07	0.85	8.00	14.00	19.75
	E2J-4	11.50q	1.51	0.62	10.00	14.00	13.19
	E3J-2	12.17abz	2.23	0.91	9.00	14.00	18.32
	K-J	16.67xyab	2.73	1.12	13.00	20.00	16.40
	K+ J	2.50xzqw	0.55	0.22	2.00	3.00	21.91
Infestation prior to the treatment in the second year	E1J'-9	19.00x	1.09	0.45	17.00	20.00	5.77
	E2J'-4	22.00y	1.79	0.73	20.00	25.00	8.13
	E3J'-2	21.67z	2.73	1.12	18.00	24.00	12.61
	K-J'	22.50q	2.43	0.99	19.00	26.00	10.80
	K+J'	6.17xyzq	1.17	0.48	5.00	8.00	18.96
Infestation following the treatment in the second year	E1J'-9	15.83xabn	1.33	0.54	14.00	18.00	8.39
	E2J'-4	20.00ywa	1.79	0.73	18.00	23.00	8.94
	E3J'-2	19.67zmb	3.20	1.31	15.00	23.00	16.29
	K-J'	26.50xyzq	2.35	0.96	23.00	30.00	8.85
	K+J'	0.67qwmn	0.82	0.33	0.00	2.000	12.47
Infestation prior to the treatment in the third year	E1J"-9	23.50xqm	1.05	0.45	22.00	25.00	4.46
	E2J"-4	29.50yamn	1.38	0.56	28.00	31.00	4.67
	E3J"-2	33.00qwao	1.79	0.73	30.00	35.00	5.42
	K-J"	34.83xyz	2.56	1.05	31.00	38.00	7.36
	K+J"	7.67zwno	1.37	0.56	6.00	9.00	17.82
Infestation following the treatment in the third year	E1J"-9	20.67xwnr	1.36	0.56	19.00	23.00	6.61
	E2J"-4	27.17yno	1.72	0.70	25.00	29.00	6.34
	E3J"-2	30.00zwm	1.79	0.73	27.00	32.00	5.96
	K-J"	65.67xyzq	2.58	1.05	62.00	69.00	3.93
	K+J"	2.83qmor	1.17	0.48	1.000	4.000	41.26

Same letters denote significant difference*:*
x, y, z, q, w, m, n, o, r; $p<0.001$
a, b, c, d; $p<0.01$

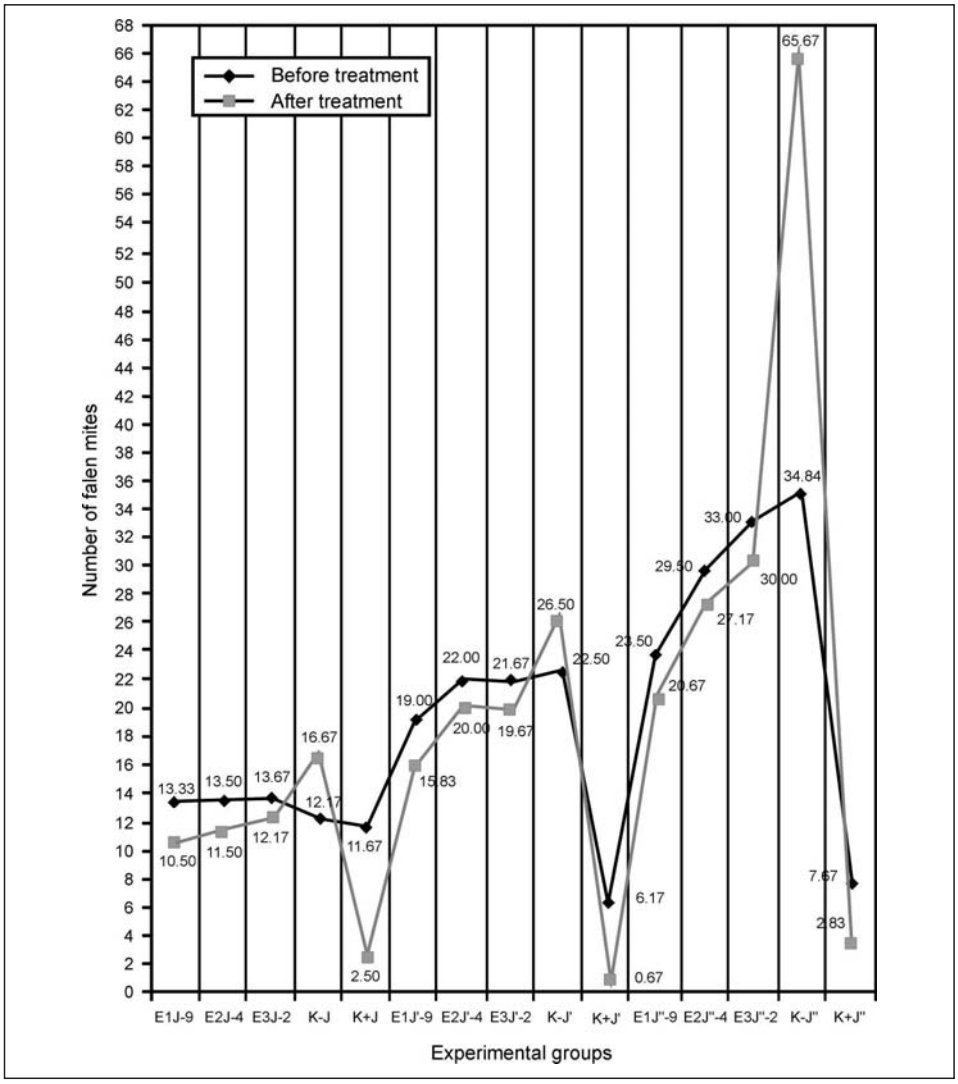

Figure 1. Levels of infestation of bee colonies with *Varroa destructor* prior to and following the treatment in three successive years assessed by sugar shake method for the assessment of levels of infestation

In the third year all groups remained homogenous after the treatment excepting the positive control, which was attributed to the application of flumethrine in previous two consecutive years. The highest average number of fallen mites was in the negative control (65.67), resulting from the absence of any anti-varroa treatment in 2006 and 2005. By statistical analysis of the results and

their comparison with the ones before the treatment it was revealed that among the treated groups the level of infestation was the lowest in group E1J"-9 (20.67) (Table 1, Figure 1). However, in comparison to the positive control all the groups treated with sugar had significantly ($p<0.001$) higher levels of infestation, but still significantly lower in comparison with the negative control ($p<0.001$). The differences between treated groups were also significant ($p<0.001$), with the exception of the one between groups E1J"-4 and E1J"- 2 (Table 1).

2. Varroa infestation levels determined with the deployment of mesh bottom boards equipped with sticky inserts

The results of investigation on the efficacy of pulverised sugar in „knocking-down" varroa mites and the influence of the dynamics of the treatment on the degree of infestation with *V. destructor* obtained with mesh bottom boards and sticky inserts are on display in Table 2 and Figure 2.

The results clearly indicate that in the first year the honey bee colonies were homogenous before the treatment, as confirmed by the coefficient of variation (Cv) which was lower than 33%, i.e. which ranged from 24.52 to 35.51. The degree of infestation in all strong colonies was roughly similar, ranging from 0.67 to 2.00 fallen mites on average (Table 2).

Some significant ($p<0.05$) intergroup differences in infestation levels were present. Tukey's test confirmed their presence ($p<0.05$) only between E1J-9 and E2J-4 treatment groups, but not between the remaining ones (Table 2). There results enabled the assessment of the effect of pulverised sugar, i.e. the comparison of levels of mite infestation before and after the treatment, between groups and comparison with the controls.

As displayed in Table 2 in the first year the experimental groups remained homogenous ($Cv<33\%$) after the treatment. Having compared the number of fallen mites prior to and following the application of sugar dust it becomes noticeable that the treatment had certain effects. The best effect in knocking down and lessening the number of mites was produced when dusting was performed at three-day intervals (Figure 2), which was proved by the increase in the average number of mites fallen to the sticky insert in E1J-9 before treatment from 2.00 to 95.17 after completion of the treatment. With the exception of group E1J-9, the decrease in the degree of infestation was also noticed in two more treated groups, E2J-4 and E3J-2, but the steepest decline was observed in the positive control (from 1.33 mites on average before to 1498 after the treatment, Figure 2).

Significant ($p<0.001$) intergroup variation in levels of *Varroa infestation* (Table 1) were detected. Tukey's test showed significant differences in infestation levels among all compared groups. All treatment groups (E1J-9, E2J–4 and E3J-2), as well as negative control (K-J) had significantly ($p<0.001$) lower infestation levels compared to the positive control (K+J). Significant ($p<0.01$) differences were noticeable between treated groups and between each treated group and the negative control (Table 2, Figure 2).

In the second year the analysed bee colonies were homogenous before the treatment, as was characterised by Cv, which was below 33%, i.e. ranged from

24.52 to 32.06. The degree of infestation in all colonies described through the number of fallen mites ranged from 1.83 to 9.17 (Table 2, Figure 2).

There were significant ($p<0.001$) intergroup differences in levels of *Varroa* infestation (Table 2). Tukey's test revealed the highest significant ($p<0.001$) differences between the negative control (K-J') and treatment groups E1J'-9 and E3J'-2, and somewhat less significant ($p<0.01$) between the negative control and E2J'-4.

As it is noticeable in Table 2 the bee colonies remained homogenous following the treatment in the second year (Cv<33%). Sugar dusting affected the mite fall-off most significantly if repeated every other day.

In group E1J'-9 the mean number of fallen mites onto a sticky insert increased from 8.67 before to 614.50 following the treatment, which lead to the conclusion that the best effect in knocking-down mites and lessening their population in the hive was obtained after sugar dusting at three-day intervals (Figure 2). In addition to this, an appreciable effect of sugar dusting was gained following the treatment with sugar in the remaining two groups (E2J'-4 and E3J'-2), although the most pronounced decrease in the level of infestation was noticed in the positive control (from 5.33 fallen mites on average prior to to 1519 mites following the treatment).

Following the treatment, significant ($p<0.001$) group differences in levels of Varroa infestation were revealed (Table 2). Tukey's test confirmed that in all the three groups treated with sugar and the negative control, the numbers of fallen mites were significantly ($p<0.001$) lower in comparison with the positive control. In comparison to the negative control significant differences ($p<0.01$ and $p<0.001$) were observed in comparison to the group E1J'-9 and the positive control (K+J'), respectively.

In the second year the homogeneity of the experimental groups before the treatment was confirmed by the coefficient of variation, which was 23.45. The level of infestation in all strong bee colonies in the research was roughly similar, being 3.50 fallen mites on average (Figure 2).

According to the results of ANOVA there were no significant ($p<0.05$) intergroup differences in the levels of infestation (Table 2), rendering Tukey's test unnecessary.

As displayed in Table 2 the homogeneity of the experimental groups remained undisturbed after the treatment (Cv<33%). Dusting bees with sugar affected the number of fallen varroa mites.

The most powerful effect in knocking-down mites and lessening the ectoparasite burden in the hives was brought about after the application of sugar at three-day intervals (Figure 2); the conclusion was based on the fact that in group E1J"-9 the average number of fallen mites onto the bottom board following the treatment increased from 9.67 to 1278.50. Tukey's test detected no significant differences between E1J"-9 and the positive control, which indicates that dusting with sugar (ground to particles lesser than 40 μm in diameter) at three-day intervals under certain circumstances may have a similar effect to the one of flumethrin.

Table 2. Descriptive and comparative statistical analysis of levels of infestation of bee colonies with *Varroa destructor* assessed by the technique which involves mesh bottom boards equipped with sticky inserts prior to and following the treatment in the three-year period

Period and year of assessment	Treated group	$\bar{X}$	SD	SE	Min	Max	CV (%)
Infestation prior to the treatment in the first year	E1J-9	2.00A	0.63	0.26	1.00	3.00	31.62
	E2J-4	0.67A	0.52	0.21	0.00	1.00	27.46
	E3J-2	1.50	0.55	0.22	1.00	2.00	35.51
	K-J	1.17	0.75	0.31	0.00	2.00	24.52
	K+J	1.33	0.83	0.33	0.00	2.00	28.24
Infestation following the treatment in the first year	E1J-9	95.17xabc	5.19	2.12	87.00	102.00	5.46
	E2J-4	45.00yade	2.36	0.96	42.00	48.00	5.26
	E3J-2	21.00zbdf	3.09	1.26	17.00	24.00	14.75
	K-J	3.667qcef	1.03	0.42	2.00	5.00	28.17
	K+J	1498.00xzyq	539.80	20.4’	910.0	2403.00	36.05
Infestation prior to the treatment in the second year	E1J’-9	8.67y	2.42	0.98	4.00	11.00	27.95
	E2J’-4	7.33a	1.21	0.49	6.00	9.00	16.51
	E3J’-2	9.17xA	3.76	1.53	5.00	15.00	32.06
	K-J’	1.83xya	0.75	0.31	1.00	3.00	31.06
	K+J’	5.33A	1.03	0.42	4.00	7.00	19.36
Infestation following the treatment in the second year	E1J’-9	614.50xaA	11.90	7.17	284.0	781.00	27.97
	E2J’-4	283.70y	8.99	3.47	126.0	347.00	28.91
	E3J’-2	154.00zA	3.36	5.45	140.0	175.00	8.67
	K-J’	6.50qa	2.43	0.99	3.000	9.00	35.37
	K+J’	1519.00xyzq	20.6	12.5	853.0	2281.00	34.28
Infestation prior to the treatment in the third year	E1J”-9	9.67qw	1.87	0.76	1.00	6.00	23.45
	E2J”-4	7.50xma	1.87	0.76	1.00	6.00	23.45
	E3J”-2	11.50xyz	1.87	0.76	1.00	6.00	23.45
	K-J”	3.67yqma	1.87	0.76	1.00	6.00	23.45
	K+J”	4.33zw	1.87	0.76	1.00	6.00	23.45
Infestation following the treatment in the third year	E1J”-9	1278.00wqm	1.40	4.99	1136.00	1420.00	7.86
	E2J”-4	607.50xwna	5.51	2.66	532.00	693.00	9.14
	E3J”-2	296.30yqab	8.98	1.83	264.00	343.00	9.78
	K-J”	27.83zmnb	6.37	2.60	21.00	37.00	22.88
	K+J”	1447.00xyz	4.20	9.10	1015.00	2124.00	28.62

Same letters denote significant difference:
x, y, z, q, w, m, n, o, r - $p<0.001$; a, b, c, d -$p<0.01$; A, B,C - $p<0.05$

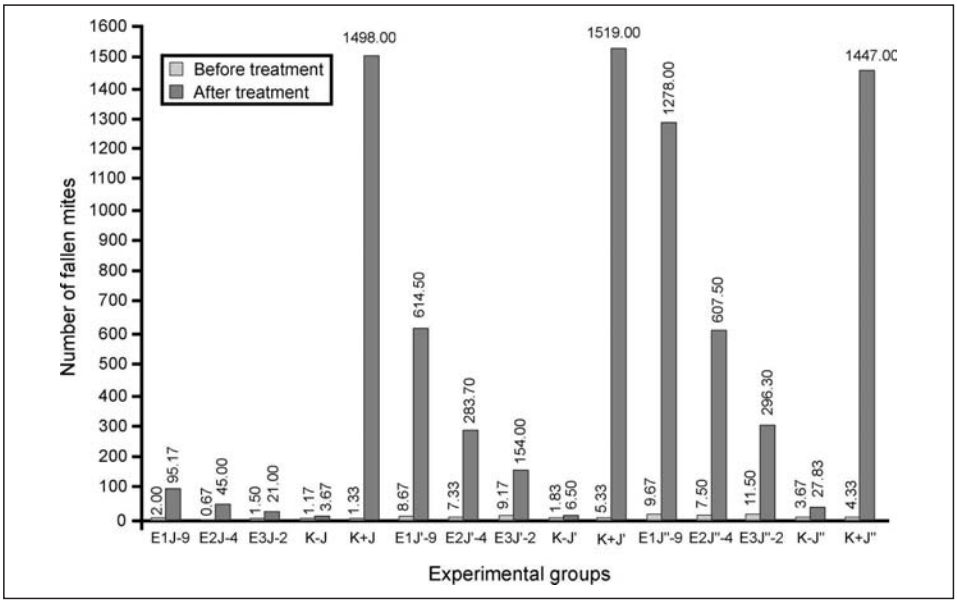

Figure 2. Levels of infestation of bee colonies with *Varroa destructor* prior to and following the treatment in three successive years assessed by the technique which involves mesh bottom boards equipped with sticky inserts

DISCUSSION

Varroa mites have been considered to pose problems in beekeeping for about 40 years. Despite the fact that due to intensive research our knowledge on the mite distribution, pathogenesis, host–parasite interactions and the effective use of certain treatments substantially increased in last decades, a solution for the infestation is yet to be proposed, which render further research on mite biology, tolerance breeding, and *Varroa* treatment absolutely necessary (Rosenkranz *et al*., 2010).

There is no absolutely effective chemical treatment. Those killing susceptible individuals leave more resistant mites to reproduce, rendering the varroa population increasingly resistant over time. Natural substances such as oxalic acid and thymol have not been proved to induce resistance, but whilst they reduce mite populations, they are not consistently highly effective. The lack of effective substances for the control of *Varroa* mites allows the ectoparasite populations to grow to injurious levels triggering colony collapse directly due to the high number of mites, or indirectly, decreasing bee immunity and favouring viral infections. Moreover, *Varroa* resistance to acaricides lead to the highly frequent applications, increased doses and residues in the hive (Le Conte *et al.*, 2010), resulting in contamination of bee products with unwanted consequences

on consumers' health (Stanimirovic *et al.*, 2003a, 2003b, 2005a, 2007a; Stevanovic *et al*., 2008).

The results of the current work proved that there was certain efficacy of the treatment with powdered sugar (particles of diameter below 40 μm) in knocking-down *V. destructor*, since in all bee colonies treated (at each dynamics and in all years) a decline in the level of infestation was noticeable in comparison to the level prior to the treatment, which is in accordance with the results of Fakhimzadeh (2000). In addition, there were intergroup differences between the groups treated with sugar, mostly significant ($p<0.001$ or $p<0.01$), which indicates the effect of the dynamics of the treatment. The most powerful effect on mite knocking-down and consecutive decrease in their population in the hive was produced by sugar dusting performed at three-day intervals, not unlike it was previously proved by Fakhimzadeh (2000). However, the same author (Fakhimzadeh, 2001b) in an experiment on worker bees placed in a glass container and treated with 5 g and 0.5 g powdered sugar proved the high efficacy of sugar resulting in 91% and 62% fallen mites, respectively. Our results also point to the efficacy of the treatment of hives powdered with 40 g of pulverised sugar (particles lesser than 40 μm) in knocking-down mites. It is to emphasize that the efficacy of such treatment was far lower, being 87.2% on average assessed by sugar shake method and 68.6% by the technique which involves mesh bottom boards.

Mite fall resulting from the effect of powdered sugar in our work was significantly ($p<0.001$ or $p<0.01$) higher in comparison to the negative controls. These findings are in accordance with those of Macedo and Ellis (2002), and Fakhimzadeh (2000, 2001b) who reported on sugar dusting which resulted in high efficacy in knocking-down mites (>90% fallen mites). However, the efficacy of sugar dusting in knocking-down mites in our work was continuously lower (68.6-87.2%) in comparison to those of flumethrin as positive control (92.5-95.5%, Cirkovic *et al*., 2011). Given all data, treatment with sugar (particles up to 40 μm) at dynamics and doses described may not be advisable as the only mean of combat against *V. destructor* due to the absence of consistence in efficacy in all situations. Throughout the experiment no adverse affects on the development of sealed brood and increase in the bee population were noticed neither in the hives dusted with sugar, which is in accordance with the findings of Fakhimzadeh (2001c), nor in the ones treated with other dust materials (Loglio and Pinessi, 1993; Macedo and Ellis, 2002). Moreover, no adverse effects of the sugar on queens (no lethality or replacement) were noticed, which was also in accordance with Fakhimzadeh (2001c). Therefore, this ecological method meets all the criteria for implementation into integrated pest management strategies to control *V. destructor* (Stanimirovic *et al*., 2007b). The treatment with sugar dust applied in a way described in the work may be used as means of combat against varroa in combination with biotechological measures or organic acids and aetheric oils in organic bee keeping. The efficacy of biotechnological measures, organic acids and aetheric oils is approximately 50%. Thus with complementary activity of these measures (building frame, bait-frame) and sugar dusting, or complementary co-action of organic acids and aetheric oils with powdered sugar (particles' size below 40 μm) at the dynamics and dose applied in this research, the efficacy of

mite knocking-down may be even beyond 97% (Goodwin and Van Eaton, 2001; Stanimirovic *et al*., 2007b; Cirkovic *et al*., 2011). This assumption is also supported by the fact that in certain circumstances, when applied nine times a month, i.e. at three-day intervals, sugar dust may be as effective as flumethrin (Cirkovic, 2011).

It is to bear in mind that there are certain differences in tolerance mechanisms towards *Varroa* mites between various subspecies of *A. mellifera*, as well as between sub-populations of the same subspecies from various climatic regions. Differences in virulence and reproductive capabilities of various haplotypes of *Varroa* contribute to differences in the parasite-host relationship between bees and mites (Navajas *et al.*, 2010; Rosenkranz *et al*., 2010). Given that the results of the current research were obtained solely on bees of Sjenica-Peshter ecotype, which differs in the expression of active behavioral defences from other bees of the same species (Cirkovic, 2002; Stanimirovic *et al*., 2002, 2003c, 2005b, 2008, 2010; Stevanovic, 2007), the effect of the treatments applied in this wotk may be somewhat different in other bees of *A. mellifera* species.

In addition, it is noticed that the technique involvings mesh bottom boards equipped with sticky inserts proved more efficacious and sensitive in the assessment of the level of infestation in bee colonies with *Varroa* destructor in comparison to the sugar shake method applied on 300 bees dusted with 40 g sugar (particle size below 40 μm).

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UTICAJ TRETMANA ŠEĆEROM U PRAHU NA STEPEN INFESTIRANOSTI PČELINJIH ZAJEDNICA EKTOPARAZITOM *Varroa destructor*

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SADRŽAJ

Cilj rada bio je da se ispita efikasnost primene šećernog praha u obaranju krpelja *Varroa destructor* i uticaja dinamike tretmana na stepen infestiranosti pčelinjih zajednica. Za procenu stepena infestacije pčelinjih društava krpeljima *V. destructor* korišćene su dve metode: metoda rolovanja sa šećerom i metoda žičane podnjače sa lepljivim ulošcima. Istraživanje je obavljeno na 30 jakih pčelinjih zajednica tokom tri godine.

Rezultati su ukazali da je zaprašivanje prah šećerom čestica dijametra ispod 40 μm imalo efekat u obaranju krpelja *V. destructor*, u odnosu na stanje pre tretmana i u odnosu na negativnu kontrolu. Najbolji efekat u obaranju krpelja i smanjenju populacije krpelja u košnici imala je primena prah šećera svakog trećeg dana. To znači da je dinamika tretmana uticala na stepen infestiranosti pčelinjih zajednica. Međutim, efikasnost metode zaprašivanja šećerom u prahu bila je značajno manja u odnosu na efikasnost flumetrina (pozitivnu kontrolu). Stoga se tretman prah šećerom nikako ne može preporučiti pčelarima kao jedina mera zaštite pčelinjih zajednica od ektoparazita *V. destructor* i pored uočenih pozitivnih efekata na smanjenje stepena infestiranosti tim krpeljom.

Korišćenje žičane podnjače sa lepljivim ulošcima predstavlja mnogo efikasniji i osetljiviji metod za procenu stepena infestiranosti pčelinjih zajednica Varroa krpeljom u odnosu na metod rolovanja u tegli sa 300 pčela posutih sa 16 grama šećera u prahu sa česticama žiji je dijametar manji od 40 μm.