VarroxSan™

Efficacy Trials Summary

SUSTAINED-RELEASE FIBER STRIP FOR CONTROL OF VARROA MITES ON BEES

Product Identification

Product Name: VarroxSan (EPA Reg. No. 94413-3)

Active Ingredient: Oxalic Acid Dihydrate (18.42%)

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Overview

Introduction

On December 01, 2023, the US EPA registered VarroxSan (EPA Reg. No. 94413-3) a sustained-release fiber strip designed for use in beehives to control the parasitic mite *(Varroa destructor)* on honeybees. The active ingredient in VarroxSan is Oxalic acid Dihydrate at a concentration of 18.42%.

VarroxSan is applied one strip per 2.5 Frames of Bees (FoB). The appropriate number of strips are taken from the pack, folded in half and each strip is hung over one comb frame inside the brood area or the bee cluster, with a minimum distance of 2 frames between strips, for example, one strip over frames 2, 4, 6 and 8. VarroxSan strips are hung in the brood chamber in such a way that the bees can walk on the strips, slightly away from the surface of the frames, and left inside the hive for 42 days to 56 days, before removal. If after 42 days bees are not in contact with the strips, the strips are repositioned closer to the cluster. Strips must be removed after a maximum of 56 days.

VarroxSan strips can be used in the spring-summer and/or the fall if varroa mite infestations have reached treatment threshold.

Pharmacodynamic properties

Oxalic acid dihydrate is an organic acid used for the treatment of *Varroa destructor.* Studies on the exact mode of action of oxalic acid are not available. However, it is believed that the major contributor to the acaricidal effects is the low pH of the dispersion, as opposed to the volume of dispersion administered. It acts as a contact poison against *Varroa destructor.*

Slow-release strips, as demonstrated by different researchers in many of their publications, have proven to be one of the best pharmaceutical forms available for the application of acaricides in beekeeping. Considering that most of the available drugs act by contact and that the parasite life cycle includes a reproductive stage within the brood cells (where most acaricides are ineffective), it is important that the applied product acts inside the hive for a time equivalent to, at least, three lifecycles of the parasite (30- 45 days), in order to maximize the probability of contact of the product with as many mites as possible, thus achieving adequate control of its population.

As an alternative, other pharmaceutical forms require repeated applications at regular and short time intervals, but that is economically unfeasible in certain farms given their production scale or mobility costs. Because of the above, and its easy application as a ready-to-use product, slow-release strips are (and probably will be for much longer) the best alternative as a pharmaceutical form for the application of acaricides in beekeeping production.

Pharmacokinetic particulars

There is evidence that oxalic acid dihydrate dispersions can penetrate keratin, because a short time after application the concentration of oxalic acid dihydrate is slightly increased in all bee tissues.

Oxalic acid dihydrate is externally distributed on the bees through body contact and/or social food exchange (trophallaxis). The active substance will also be delivered to any Varroa mites that are attached to or in close proximity to adult bees.

VarroxSan, like other registered strip products such as Apivar (Amitraz) and Apistan (Tau-Fluvalinate), works independently of the external environment, unlike acaricidal products based on Thymol and Formic acid.

Honeybee Safety

Although honeybees are more tolerant to oxalic acid dihydrate (OAD) than mites, they do exhibit some negative effects, especially at high concentrations. When treated with doses insufficient to directly cause mortality (sublethal doses), bees experience decreased activity and changes in their exoskeleton cuticle enzymes. Doses large enough to cause mortality cause internal tissue damage (Martín-Hernández, et al. 2007).

Some reported effects show that bees exhibit behavioral changes when drip-treated in sublethal doses of OAD. At 175µg of OAD / bee, Schneider et al. (2012) found that the treatment caused a decrease in worker bee activity, bee nurse behavior and longevity, yet despite these unwanted effects this treatment caused an increase in cleaning behavior. Neurotoxic damage was suspected to be a possible cause of behavior change based on similar results from another study in which treated bees were immobilized and did not regain mobility. However, the authors also recognized that changes in the behavior of the bees could have resulted from damage to the digestive and excretory organs, leading to insufficient reabsorption of nutrients through the gut epithelium and the resulting weakening of the bees. For comparison, the dose used in this study was approximately three times higher than the typical maximum dose of 57µg OAD / bee. For its part, VarroxSan, applied at the recommended dose, contains 16.3µg OAD/bee/day.

The strength of the colonies is estimated using the number of frames of bees. A standard Langstroth honeycomb covered with a layer of bees contains approximately 1,900 bees per frame side. Based on this calculation by Kauffeld (1975), cited by Fries et al. (1991), the concentration or dose of the miticide product evaluated was determined. Thus, for VarroxSan: 2.5 frames of Bees: 9,500 - 1 strip of VarroxSan 7g Oxalic acid Dihydrate (7,000 mg). Total = 7,000/9,500 = 0.736mg; 0.736/45 days of treatment: 0.0163mg or 16.3 µg OAD/bee/day.

In all reviewed studies, bee safety was taken into consideration but only using the recommended dose. No differences were found either in bee population or brood between control hives (untreated) and positive control hives (registered products).

One study was performed using 1.5 times the recommended dose by Dr. Alexandros Papachristoforou of Aristotle University see page 9.

The field study evaluated 3 different treatments:

1. Untreated control
2. 4 strips per hive (label rate)
3. 6 strips per hive (1.5X label rate)

The study conclusions are:

Both applications of VarroxSan (4 strips and 6 strips) appeared very effective against the mites of *Varroa destructor.* The pest was radically reduced from the colonies which initially were heavily infested. Colony strength was normal and the product appeared to be tolerated by honeybees since no increased mortality was observed and no queen was lost. Control (untreated) colonies presented higher losses and reduced strengths when compared to the experimental colonies.

Higher dosing of VarroxSan (6 strips) induced no significantly higher mortality to bees and no lower strength when compared to VarroxSan label-rate dosing (4 strips).

No behavioral changes were observed at any of the experimental colonies and no queen was lost. No effect was observed on brood and queens were observed to lay eggs even close to the VarroxSan strips.

In conclusion, VarroxSan applied in colonies for 56 days, appears to be an excellent way of controlling varroa infestation, furthermore, both normal dose (4 strips) and 1.5 times the dose (6 strips) were safe for the honeybees.

Clinical documentation

Hive structure

In Greece, Argentina, Uruguay and the USA including California, the type of hive used is Langstroth hives.

Moreover, products like Apivar and Apistan have the same posology registered in Spain, Italy, France and USA where the type of colony used is different (Layens hives in Spain; Dadant hives in Italy and France), meaning the colony structure has no impact on the posology and in the final product performance.

The influence of climate on pest behavior

The internal temperature and humidity of bee hives are kept relatively constant with little variability. A varroa treatment applied inside the hive will be exposed to the same conditions regardless of the region of the world where the hive is located.

Even though this type of product will not be directly influenced by the external temperature, VarroxSan was tested in 6 different regions (4 countries) but all of them have a similar beekeeping season. At the end of the summer (honey production), bee colonies naturally will start preparing themselves for the winter period. Nowadays, this is artificially induced by beekeepers. Weather does not have a direct impact on the pest behavior. The survival of the hives will depend on the varroa treatment performed at the end of summer.

Varroa mite resistance worldwide

There is no known resistance to Oxalic acid in varroa populations worldwide. In Argentina, Maggi et.al (2016) concluded: "The results reported here suggest that the Varroa population exposed during 8 successive years to oxalic acid treatments remains susceptible to this acid."

Summary of the trials

The 6 studies reviewed here were performed during late summer and early autumn. In all trials, VarroxSan treatment efficacy was significantly better than the control.

A short narrative of each of the trials, which includes experimental design, treatments, results, and statistical analyses conclusions can be found in Table 1.

Table 1: The following table is a basic summary of the study type, treatments, and length of observation.

Study location	Study type	Treatments (hives)	Duration	Initial varroa infestation	Efficacy
Uruguay #1	Field efficacy	VarroxSan: 11 Control: 10	41 days	VarroxSan: 3.4% Control: 2.4%	VarroxSan: 95.3%
Argentina	Field efficacy	VarroxSan: 10 Control: 5	44 days	VarroxSan: 2.1% Control: 2.0%	VarroxSan: 97.7%
Greece	Field efficacy and Safety	VarroxSan: 10 Control: 10	56 days	VarroxSan: 2.7% Control: 2.9%	VarroxSan: 95.4%
Uruguay #2	Field Residue	VarroxSan: 8 Control: 8	58 days		
Study location	**Study type**	**Treatments (hives)**	**Duration**	**Initial varroa infestation**	**Final varroa infestation**
Michigan State University	Field efficacy	VarroxSan: 10 EPA approved Formic Acid pads: 10 oxalic acid in glycerin (RO): 10 ½ strength OA in glycerin (RO2): 10	55 days	VarroxSan: 2.6% EPA approved Formic Acid pads: 3.9% RO1: 2.8% RO2: 1.6%	*VarroxSan: 0.9% EPA approved Formic Acid pads: 4.04% RO1: 1.2% RO2: 4.9%
Washington State University	Field efficacy	VarroxSan: 12 Apivar: 12	60 days	VarroxSan: 2.30% Apivar: 2.33%	*VarroxSan: 0.24% Apivar: 5.58%

*Efficacy was not calculated. Only initial vs final infestation of varroa on adult bees was presented.

Study Location: Uruguay #1

Study Name: Control of *Varroa destructor* in colonies of bees (Apis melliferas) with Oxalic acid in cardboard strips (VarroxSan)

Testing facility: INIA La Estanzula Experimental Station, Colonia, Uruguay.

Test year: May - August, 2017

Experimental design: Field study – The study comprised 11 beehives treated with VarroxSan following label posology (1 strip every 2.5 frames covered by bees) and 10 beehives untreated as control. Colonies received an initial assessment including the following data: queen presence, number of frames of bees, number of frames of brood, and an alcohol wash sample for Varroa quantification and absence of any disease (apart from Varroa). Colonies were equipped with mesh floors and sticky cards to collect mites. Moreover an underbasket trap was placed in from of the beehives to count dead bees. After 15 and 41 days of treatment a new assessment was performed in all colonies. Finally at day 41 a follow up treatment was applied to evaluate the efficacy of the product.

Statistical analysis was performed.

Treatments:

Treatment	Tradename (EPA Reg. No.)	Report name (active ingredient)	Dose
1	VarroxSan (94413-3)	Oxalic Acid Dihydrate (18.42%)	1 strip every 2.5 frames covered by bees*
2	Control	Untreated	

** Label Use*

Results:

Comparison of the state of the colonies prior to treatment, 15 days after application and at the end of treatment (41 days after application). Average ± standard deviation of the population (honeycombs covered by bees) and brood (squares full of brood).

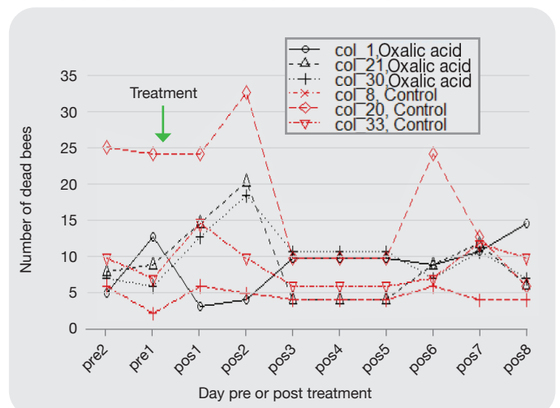
Treatment	Hives	Adult bees			Brood	
		Pre treatment	15 days post	41 days post	Pre treat	41 days post
VarroxSan	11	6.3 ±1.4	6.2 ±1.6	6.2 ±1.9	0.6 ±0.6	1.9 ±1.1
Control	10	6.9 ±1.9	7.1 ±1.9	7.4 ±1.4	0.6 ±0.4	2.3 ±1.6

Comparison of the infection level of the colonies, measured as the percentage of infection of adult bees), 15 days after application and at the end of the treatment (41 days after application). Average ± Standard Deviation.

Treatment	Hives	% of varroa on adult bees		
		Pre treatment	15 days post	41 days post
VarroxSan	11	3.4 ±2.4	0.7 ±0.5	0.1 ±0.2
Control	10	2.4 ±2.0	4.2 ±2.0	4.0 ±2.1

At 15 days post-treatment, the percentage of bees infected with mites decreased markedly in the treated group, this difference being statistically significant (W = 10 and p = 0.0083). At 41 days post-treatment, the percentage of infection by *V. destructor* continued to decrease, the difference between the treated and control colonies being significant (W = 1 and p = 0.0001).

Dead bees collected in the "underbasket" traps placed in front of the hive. Data recorded in three hives treated with oxalic acid and three control hives



The records of dead bees collected in the "underbasket" traps placed in front of the runner of three hives treated with oxalic acid and three hives without treatment (control). Both groups of hives showed similar bee mortality, suggesting that the treatment has no effect on this parameter.

Varroa fall due to the effect of treatment (in the 41 days of treatment duration) and due to the effect of chemical shock (Amitraz + Flumethrin) applied at the end of the treatment.

Hive	Natural fall pre treatment	Varroa fall during treatment	Residual mites	Efficacy (%)	Corrected efficacy (%)
1	6	2031	26	98.7	98.2
4	1	379	17	95.7	94.1
9	2.5	587	5	99.2	98.9
10	1.5	881	18	98.0	97.3
11	2	452	8	98.3	97.6
15	4	553	10	98.2	97.6
17	1	92	10	90.2	86.6
18	1.5	309	20	93.9	91.7
21	6	1004	31	97.0	95.9
26	4.5	1189	45	96.4	95.0
30	3.5	754	26	96.7	95.4
			Average	96.6	95.3
			Standard deviation	2.60	3.57

The efficacy value obtained was 96.6% ± 2.6 (mean ± standard deviation) and when correcting this value taking into account the natural mortality of the hive mites as stated by Abbot (1925), the value of the efficacy was 95.3% ± 3.57.

Conclusion:

The formulation of oxalic acid (VarroxSan) in cardboard strips is a useful and effective tool in the control of varroosis. On the other hand, no acute toxicity of the product was observed in this experiment since it had no effect on the mortality of bees.

Study location: Argentina

Study Name: VarroxSan™ Efficacy Study

Testing facility: Agricultural Experiment Station of INTA Concordia, Argentina.
Test year: May - June 2021
Experimental design: Field study – The study comprised 10 beehives treated with VarroxSan following label posology (1 strip every 2.5 frames covered by bees) and 5 beehives untreated as control. Colonies received an initial assessment including the following data: queen presence, number of frames of bees, number of frames of brood, and an alcohol wash sample for Varroa quantification and absence of any disease (apart from Varroa). Colonies were equipped with mesh floors and sticky cards to collect mites. Moreover an underbasket trap was placed in from of the beehives to count dead bees. After 44 days of treatment a new assessment was performed in all colonies. Finally at day 44 a follow up treatment was applied to evaluate the efficacy of the product.

Statistical analysis was performed.

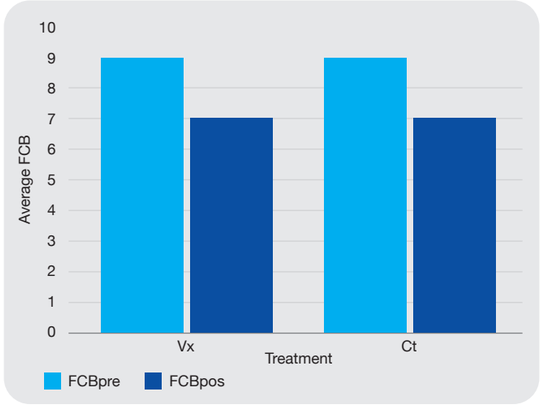
Treatments:

Treatment	Tradename (EPA Reg. No.)	Report name (active ingredient)	Dose
1	VarroxSan (94413-3)	Oxalic Acid Dihydrate (18.42%)	1 strip every 2.5 frames covered by bees*
2	Control	Untreated	

** Label Use*

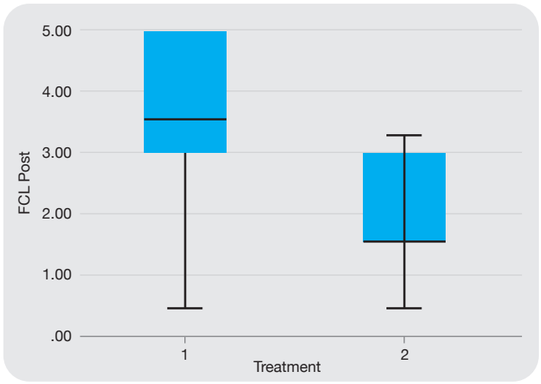
Results:

Bee population (FCB): Number of frames covered with bees (FCB) in hives that received VarroxSan™ (Vx) treatment and control hives (Ct).



At the beginning of the evaluation, the frames covered with bees (CCA) in both treatments were identical since all the hives had 9 frames. After the treatment, there were no significant differences (p = 1,000) in the population of bees, with 7 being the mean registered for both treatments.

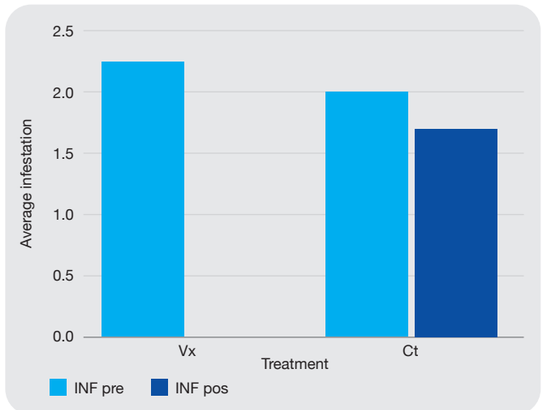
Number of frames covered with brood/larvae (FCL): Average number of frames of brood after treatment (hives that received treatment with VarroxSan™ (1) and control hives (2).



The hives that received VarroxSan™ at the end of the treatment period had significantly more brood than the control colonies, 3.55 and 1.95 FCL respectively.

Dead bees (traps)
There were no differences in the number of dead bees in the traps, with an average of 107.6 dead bees in the VarroxSan treatment and 96.8 dead bees in the control hives. Only a negligible increase in dead bees was observed in the acaricide colonies (10.8 bees).

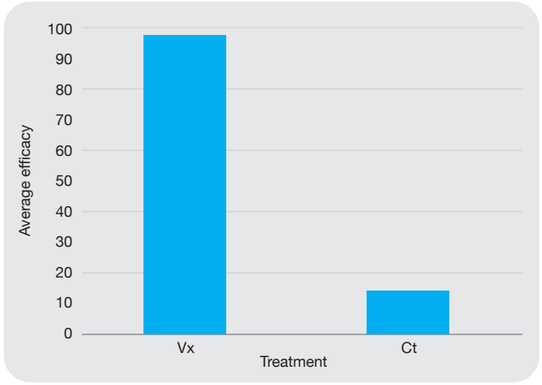
***Varroa destructor* on adult bees: Average prevalence of Varroa destructor (%) in hives that received acaricide treatment Vx and control hives (Ct).**



A significant reduction was registered in all the hives treated with VarroxSan. It should be mentioned that the percentages of infestation at the beginning of the trial were not high (Vx 2.19% and Ct 2.01%) with maximum values of 6.15% Vx and 4.03% in Ct.

The average parasitization in the two groups of hives before and after the acaricide treatment, where a significant difference is evidenced in the reduction of mites in the treated hives with respect to the control group (p = 0.001).

Efficacy: Mite count on technical floors



Significant differences were found between the treatments (p = 0.001), with an average efficacy of 98.3% ± 0.6 for VarroxSan, adjusted to 97.7% ± 0.6 after adjusting for natural mite fall according to Abbot (1925), compared with 6.3% ± 5.4 for control colonies.

The maximum percentage of efficiency obtained was 98.6% and the minimum was 96.7%.

Conclusions

- VarroxSan did not affect the adult bee population. No negative effects were observed.
- VarroxSan did not affect the number of frames with bees; the number of frames with bees did not decrease significantly.
- Higher mite mortality was recorded due to the acaricide product on the third day after being placed in the hives.
- During the 44 days of treatment, some gnawing of the support was evidenced in some hives. This was visualized at the time of making the mite counts on the technical floors and at the end of the treatment, after the supports were removed. Despite this, gnawing is not considered relevant since it did not affect the final acaricidal efficacy of the evaluated product.
- The behavior of each hive treated with VarroxSan was homogeneous. The product managed to significantly reduce the initial prevalence of mites.
- Within a strategic health management plan, its application can be advised at the end of the productive season and / or before the start of the honey season.

Study location: Washington State University

Study Name: Efficacy trial of VarroxSan for control of Varroa mites in honey bee colonies in Northwestern United States

Testing facility: Moscow, Idaho. USA.
Test date: September - November 2021
Experimental design: Field study – The study comprised 12 beehives treated with VarroxSan following label posology (1 strip every 2.5 frames covered by bees) and 12 beehives treated with Apivar (Registration Number 87243-1-AA) as positive control. Colonies received an initial assessment including the following data: queen presence, number of frames of bees, number of frames of brood, and an alcohol wash sample for Varroa quantification and absence of any disease (apart from Varroa). Colonies were equipped with mesh floors and sticky cards to collect mites. Moreover an underbasket trap was placed in from of the beehives to count dead bees. After 30 and 60 days of treatment a new assessment was performed in all colonies. Finally at day 60 a follow up treatment was applied to evaluate the efficacy of the product. Statistical analysis was performed.

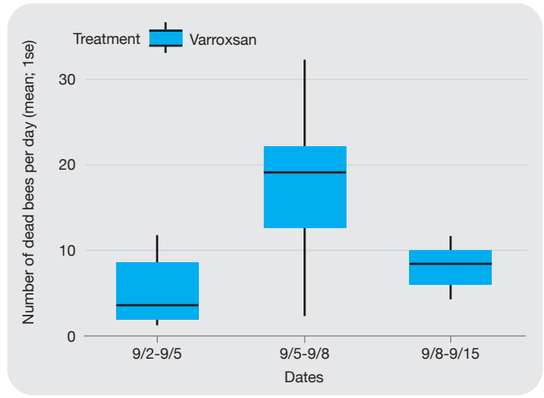
Treatments

Treatment	Tradename (EPA Reg. No.)	Report Name (Active Ingredient)	Dose
1	VarroxSan (94413-3)	Oxalic Acid Dihydrate (18.42%)	1 strip every 2.5 frames covered by bees*
2	Apivar (87243-1) Control	Amitraz 3.33%	1 strip every 5 frames covered by bees*

** Label Use*

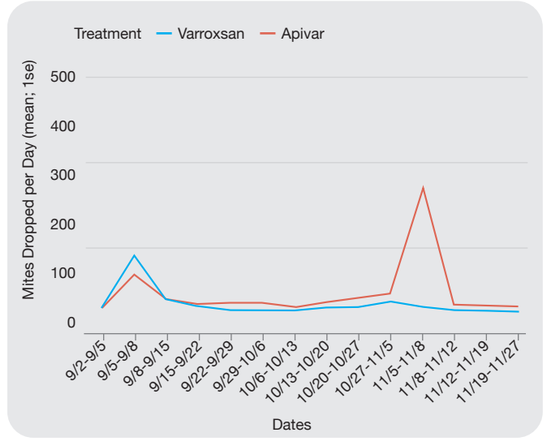
Results:

Dead bee trap analysis



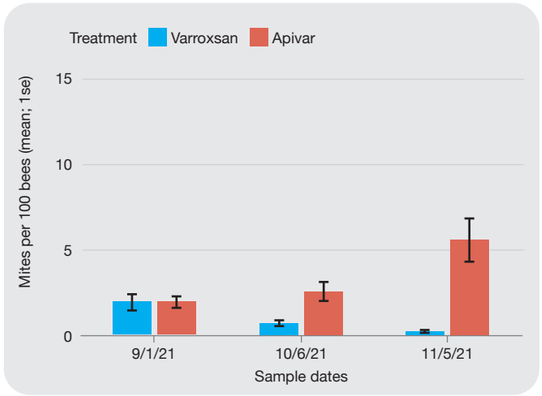
Mean daily bee mortality in hives treated with VarroxSan, as collected by dead bee trap sampling. Mean bee mortality in the first three days post treatment was 5.50±2.02 bees per day. Mean bee mortality was highest from four to six days post treatment application, with an average of 17.38±2.02 bees dying per day. In days seven to thirteen post treatment, an average of 8.71±2.14 bees died per day. In the context of an entire honeybee colony adult bee population, these numbers of dead bees should be considered biologically insignificant.

Sticky card analysis



Mean mites per sticky card +/- standard error by treatment for each sampling date. Significant difference was detected between VarroxSan and Apivar ($p < 0.05$) on November 5th, 60 days post-treatment.

Alcohol wash analysis



Per design, there were no significant differences in mean mite level between treatment groups on September 1st, before treatments were applied. Mean number of mites per 100 bees by treatment on October 6th, 31 days post-application, were as follows: VarroxSan (0.685±0.866) and Apivar (2.588±0.866). On November 5th, 60 days post-application, the mean number of mites per 100 bees for VarroxSan and Apivar, was 0.246±0.866 and 5.583±0.866, respectively. Mean mite level for VarroxSan was significantly lower post treatment than Apivar (p=0.0002)

Conclusion

Based on the results obtained, VarroxSan provided excellent control of Varroa destructor following the normal American beekeeping practices in field conditions, without any disturbance to the normal behavior of the colonies. In contrast, Apivar treatment did not provide adequate control of Varroa mites, risking the survival of the honeybee colonies during the winter. This study suggests that Varroa mite populations in this area of the United States have a degree of resistance towards the active ingredient in Apivar but not to that in the VarroxSan treatment. It seems then that VarroxSan should be an ideal treatment for beekeepers as it provides a high level of control of parasitic Varroa mites, even where mites are resistant to synthetic acaricides.

Study location: Greece

Study Name: field tests to assess the efficacy of Varroxsan™ against varroa infestation and its safety on honeybees

Testing facility: Chalkidiki, Greece.
Test date: November - March 2022
Experimental design: Field study – The study comprised 10 beehives treated with VarroxSan following label posology (1 strip every 2.5 frames covered by bees), 10 beehives treated with VarroxSan at 1.5 times the label proposed use (1 strip every 1.5 frames covered by bees), 5 beehives with blank strips (1 strip every 2.5 frames covered by bees) as control and 5 beehives with blank strips (1 strip every 1.5 frames covered by bees) as control. Colonies received an initial assessment including the following data: queen presence, number of frames of bees, number of frames of brood, and an alcohol wash sample for Varroa quantification and absence of any disease (apart from Varroa). Colonies were equipped with mesh floors and sticky cards to collect mites. Moreover an underbasket trap was placed in from of the beehives to count dead bees. After 56 days of treatment a new assessment was performed in all colonies and a follow up treatment was applied to evaluate the efficacy of the product. Statistical analysis was performed.

Treatments

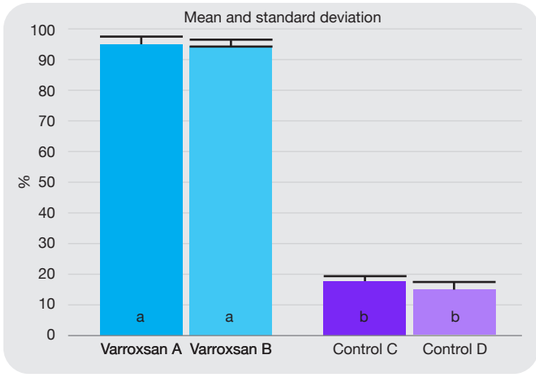
Treatment	Tradename (EPA Reg. No.)	Report name (Active ingredient)	Dose
VarroxSan A	VarroxSan (94413-3)	Oxalic Acid Dihydrate (18.42%)	1 strip every 2.5 frames covered by bees*
VarroxSan B	VarroxSan (94413-3)	Oxalic Acid Dihydrate (18.42%)	1 strip every 1.5 frames covered by bees
Control C	Control	Untreated	1 blank strip every 2.5 frames covered by bees*
Control D	Control	Untreated	1 blank strip every 1.5 frames covered by bees

** Label Use*

Results:

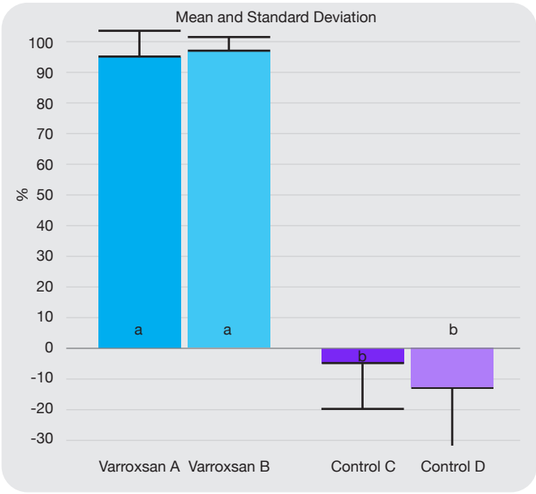
Efficacy

Figure 1: Efficacy of VarroxSan per batch and control treatments. Columns indicated by different letters (a,b) presented significant differences for p<0.05



VarroxSan presented extremely high efficacy against varroa mites (Figure 1). The average efficacy in Batch A was 95.44% (min: 93.95%, max: 97.61%) and in Batch B was 95.04% (min: 91.92%, max: 96.75%). Efficacy was significantly higher than the Control Batches (p<0.0001) as shown in Figure 1. There was no significant difference between Batch A and Batch B. In the Control Batch C, average efficacy was 18.28% (min: 16.36%, max: 20.23%), while in Control Batch D, average efficacy was 14.19% (min: 12.32%, max: 18.42%).

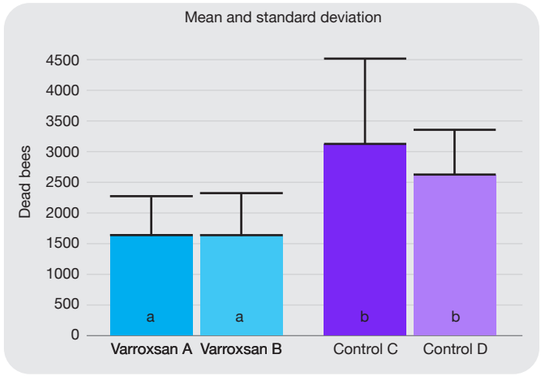
Figure 2: Efficacy of VarroxSan on Varroa mites of adult bees per batch and control treatments. Columns indicated by different letters (a,b) presented significant differences for p<0.05



VarroxSan presented also extremely high efficacy against varroa mites on adult bees (Figure 2). The average efficacy in Batch A was 96.65% (min: 74.14%, max: 100%) and in Batch B was 97.05% (min: 88%, max: 100%). Efficacy was significantly higher than the Control Batches (p<0.0001) as shown in Figure 2.

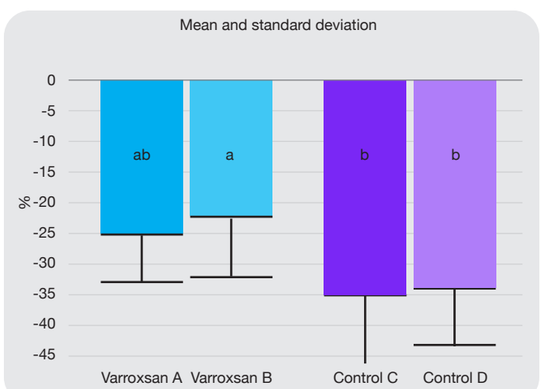
There was no significant difference between Batch A and Batch B as well as between Batch C and Batch D. In the Control Batch C, average efficacy was -6.53% (min: -28.95%, max: 11.27%), while in Control Batch D, average efficacy was -12.68% (min: -39.9%, max: 9.5%).

Figure 3: Comparison of dead bees per batch of VarroxSan™ and control treatments. Columns indicated by different letters (a,b) presented significant differences for p<0.05



The number of dead bees per colony during the application of VarroxSan is presented in Figure 3. Results showed that the mortality of bees in both applications of normal and overdose of VarroxSan (Batches VA and VB) was low and statistical analysis indicated that this mortality was significantly lower than mortality in the Control Batches (p=0.0099). This can be explained by the level of infestation. Heavily infested control colonies received no treatment for an extended period. As a result, they weakened rapidly and three of the eight colonies collapsed during the test. An overdose of VarroxSan (Batch B) resulted no significantly higher bee losses compared to single dose in Batch A. Mortality in Batch C did not differ significantly when compared mortality in Batch D.

Figure 4: Comparison of population reduction during experiments. Columns indicated by different letters (a,b) presented significant differences for p<0.05



Strength of the experimental colonies (in terms of numbers of adult honeybees) followed the normal reduction of late autumn/winter in all Batches. Reduction of population in control colonies was slightly higher (p<0.054) compared to experimental colonies (Figure 4) and followed also the normal reduction rates of late autumn/winter. Furthermore, reduction presented no significant differences between Batch A (normal dose of VarroxSan) and Batch B (overdose of VarroxSan). None of the colonies, experimental or control, collapsed during the experiments.

Conclusion

Both applications of VarroxSan (4 strips and 6 strips) appeared very effective against *Varroa destructor.* The pest was radically reduced from the colonies which initially were heavily infested. Colony strength was normal and the product appeared to be tolerated by honeybees since no increased mortality was observed and no queen was lost. Control (untreated) colonies presented higher losses and reduced strength when compared to the experimental colonies.

Higher dosing of VarroxSan (6 strips) induced no significantly higher mortality to bees and no lower strength when compared to VarroxSan label dose (4 strips).

No behavioral changes were observed at any of the experimental colonies and no queen was lost. No effect was observed on brood and queens observed to lay eggs even in close proximity to the VarroxSan strips.

As a conclusion, VarroxSan applied in colonies for 56 days appears to be an excellent way of controlling varroa infestation, furthermore, both normal dose (4 strips) and 1.5 times the dose (6 strips) were safe for the honeybees.

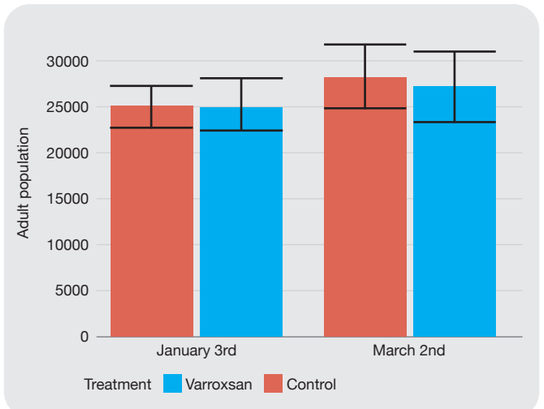
Study location: Uruguay

Study Name: Honey and beeswax quality after VarroxSan™ application in honey bee colonies during nectar flow

Testing facility: INIA La Estanzuela, Colonia, Uruguay.
Test date: January - March 2021
Experimental design: Field study – The study comprised 8 beehives treated with VarroxSan following label posology (1 strip every 2.5 frames covered by bees) and 8 untreated beehives as control. Colonies received an initial assessment including the following data: queen presence, number of frames of bees, number of frames of brood, and an alcohol wash sample for Varroa quantification and absence of any disease (apart from Varroa). After 58 days of treatment a new assessment was performed in all colonies and samples of honey and beeswax were taken and sent to the laboratory for analysis (Content of Oxalic acid, Moisture, Acidity and pH). Statistical analysis was performed.

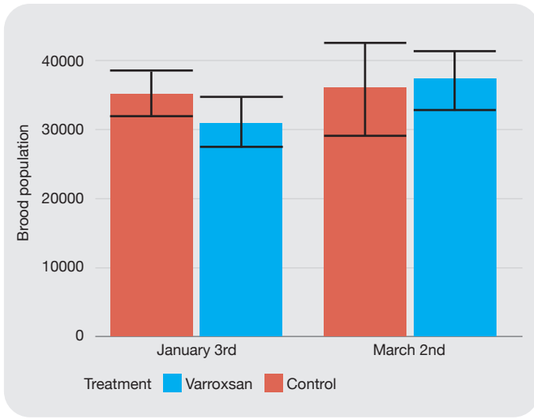
Results:

Adult bee population



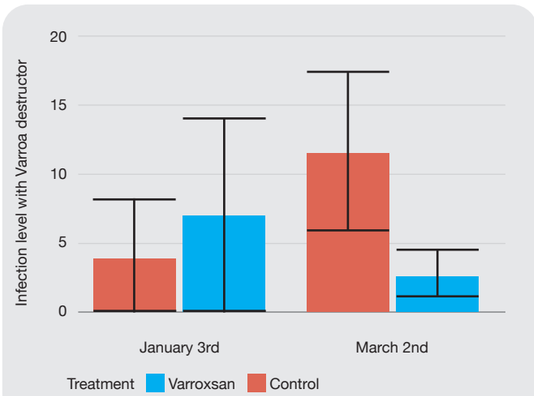
Adult bee population in control and VarroxSan treated colonies at the beginning of the experiment (January 3rd, 2022) and at the end of the experiment (March 2nd, 2022).

Brood population



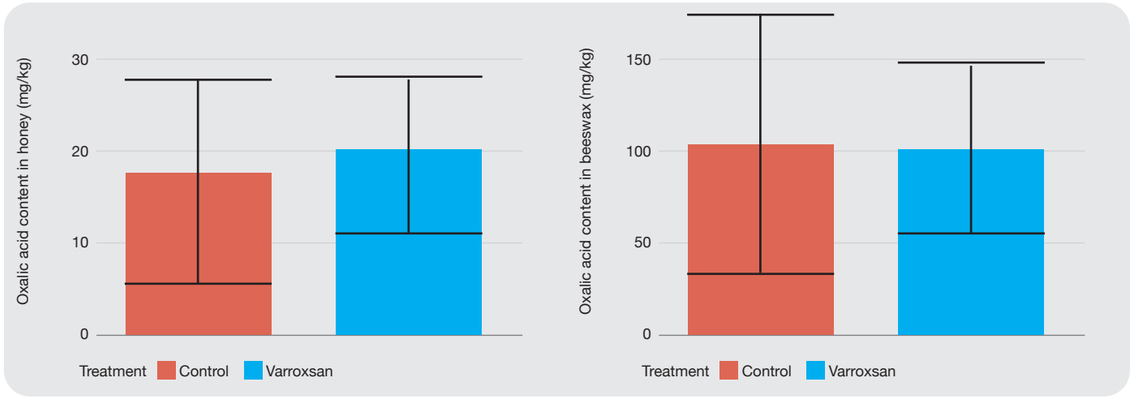
Brood population in control and VarroxSan treated colonies at the beginning of the experiment (January 3rd, 2022) and at the end of the experiment (March 2nd, 2022).

Infestation level with Varroa destructor



Infestation level with Varroa destructor in control and VarroxSan treated colonies at the beginning of the experiment (January 3rd, 2022) and at the end of the experiment (March 2nd, 2022).

Oxalic acid content in honey (a) and beeswax (b) at the end of the experiment (March 2nd, 2022).



Moisture content, pH and acidity in honey

Both groups of colonies showed similar moisture content, pH and acidity in honey at the end of the study.

Statistical results

Parameter	Date	Test	Statistic	p value
Adult population	Jan 3rd 2022	T test	t=0	0.99
	March 2nd 2022	T test	t=0.45	0.65
Brood population	Jan 3rd 2022	T test	t=2.27	0.03
	March 2nd 2022	T test	t=0.44	0.66
Varroa destructor	Jan 3rd 2022	Wilcoxon	W=20	0.23
	March 2nd 2022	Wilcoxon	W=53.3	0.003
Oxalic acid in honey	March 2nd 2022	T test	t=0.49	0.62
Oxalic acid in beeswax	March 2nd 2022	T test	t=0.04	0.96
Honey moisture content	March 2nd 2022	T test	t=0.23	0.82
Honey PH	March 2nd 2022	T test	t=0.11	0.9
Honey acidity	March 2nd 2022	T test	t=0.007	0.99

Statistical results of the comparisons of control colonies and VarroxSan treated for the different parameters analyzed at the beginning (January 3rd, 2022) and at the end of the experiment (March 2nd, 2022)

Conclusions

According to the results obtained in this study, the application of VarroxSan to honeybee colonies *Apis melifera* for control of *Varroa destructor* during the nectar flow, does not affect the content of oxalic acid, moisture content, acidity or pH of honey, nor does it affect the content of oxalic acid in beeswax.

In addition, it does not affect the quantity of brood and adult bees, and it does reduce varroa population, suggesting that it is a useful approach to reducing varroa load of honeybee colonies during honey production periods.

Study location: Michigan State University

Study Name: Evaluation of multiple formulations of long-term Oxalic acid applications for the control of Varroa destructor in honeybee (Apis mellifera) hives.

Testing facility: Michigan State University (Lansing, Michigan, US)
Test year: June - August 2023
Experimental design: Field study – The study comprised 10 beehives treated with VarroxSan following label posology (1 strip every 2.5 frames covered by bees), 10 beehives treated with EPA approved Formic Acid pads, 10 beehives treated with a homemade oxalic acid in glycerin (RO) treatment (500g Oxalic acid dihydrate crystals, 500g (400mL) vegetable glycerin), and 10 beehives treated with a homemade oxalic acid in glycerin and water, RO2 (600g Oxalic acid dihydrate crystals, 1300 mL glycerin, 1000 mL water). Colonies received an initial assessment including the following data: queen presence, number of frames of bees, number of frames of brood, and an alcohol wash sample for Varroa quantification and absence of any disease (apart from Varroa). Colonies were equipped with mesh floors and sticky cards to collect mites. After 14 and 55 days of treatment a new assessment was performed in all colonies. Finally at day 55 a follow up treatment was applied to evaluate the efficacy of the product. Statistical analysis was performed.

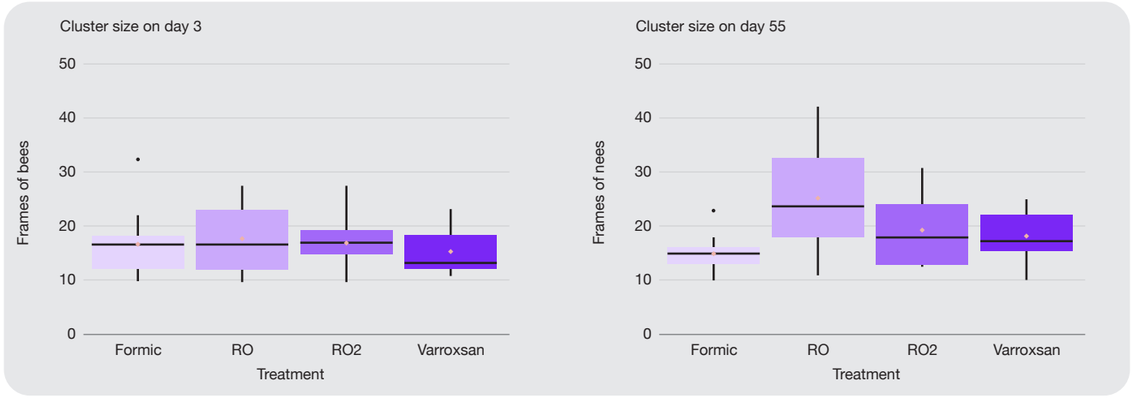
Treatments:

Treatment	Tradename (EPA Reg. No.)	Report name (active ingredient)	Dose
1	VarroxSan (94413-3)	Oxalic acid Dihydrate (18.42%)	1 strip every 2.5 frames covered by bees*
2	EPA-approved Formic acid pads (positive control)	Formic acid 42.25%	2 strips applied between two deep chambers
3	Homemade Oxalic acid in glycerin (RO	500g Oxalic acid dihydrate crystals, 500g (400mL) vegetable glycerin	two-half sponges between chambers
4	Homemade Oxalic acid in glycerin (RO2)	500g Oxalic acid dihydrate crystals, 1300 mL) vegetable glycerin, 1000 mL water	two-half sponges between chambers

** Label Use*

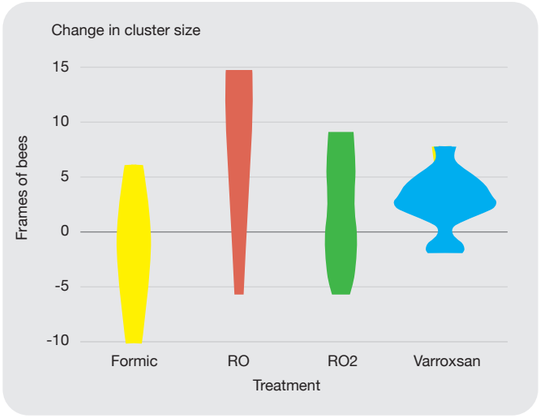
Theoretical Oxalic acid percentage
RO: 50% of oxalic acid per cellulose pad.
RO2: 29% of oxalic acid per cellulose pad.

Results:

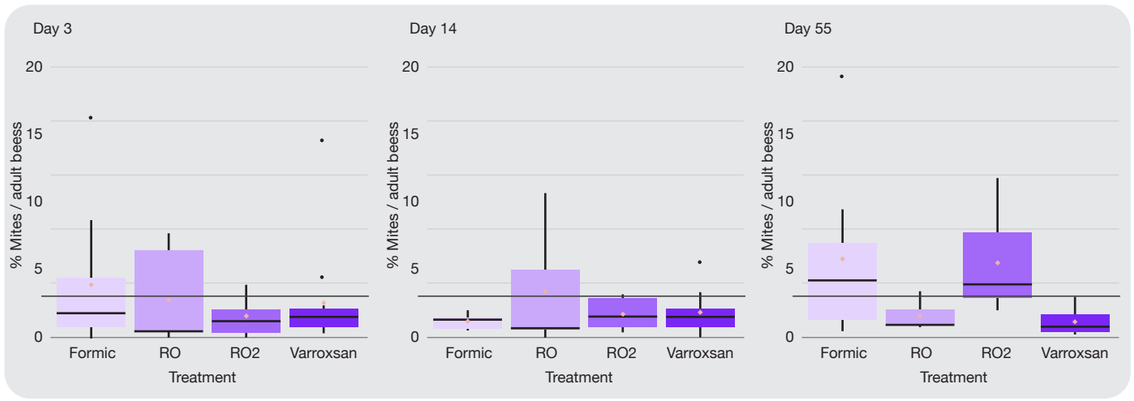


All colonies were queenright at the end of the trial. At the end of the trial, cluster sizes ranged from 10 frames of bees to 42 frames of bees, with a mean of 19.2 frames of bees. On average, the colonies receiving the formic treatments lost about 2 frames of bees in size, while the rest of the colonies grew over the trial period. One hive in the formic group was an outlier in cluster size at the start of the trial (31 frames of bees). This hive was 21 frames of bees at day 55 (losing 10 frames of bees during the trial). Even with this hive excluded, the hives in the formic group on average went down 1 frame of bees over the trial period. While the mean final cluster size (above) in the colonies receiving only formic acid was smallest (14.9; mean RO = 25.3, mean RO2=19.3, mean VarroxSan = 18.2), a Kruskal-Wallis test did not indicate any difference in cluster size among treatment groups (p=0.877), but a pairwise t-test showed that the cluster size of the colonies in the formic group was smaller than the colonies in the RO group (p=0.017).

Change in cluster size (size at day 55 – size at day -3) by treatment group. This figure shows the distribution of the change in cluster size. While the RO group had a higher average increase in frame size than the other groups, it is very widely distributed; some colonies gained 15 frames, while other colonies lost >5 frames. The colonies in the VarroxSan group had a smaller distribution, ranging from a loss of 1 frame to an increase of 7.5 frames, indicating consistent growth in this treatment group.



Mite infestation



At day 55, mean mite levels were different between treatment groups as indicated with anova (df=3, F=3.024, Pr(>F) = 0.04). Colonies who had received only one treatment of Formic acid or the RO2 formulation had over 4% mite infestation on average, whereas colonies receiving the RO formulation or VarroxSan had lower mean mite infestations (1.2% and 0.9%, respectively). **At the end of the trial, there were no colonies in the VarroxSan group that were over the 3% threshold, while all other treatment groups had at least one colony over 3%. VarroxSan was the only treatment group where mite infestation was below 1% at the end of the trial period, indicating consistent varroa control.**

Colonies with high initial mite loads

At the start of the trial, two colonies had incredibly high mite levels. One of these colonies was in the group that received the EPA-approved Formic acid pads treatment (16.3% mites) and the other was in the VarroxSan treatment group (14.6% mites). These two colonies had very different outcomes. The colony in the Formic acid treatment group became much smaller over the trial, dropping from 22 frames of bees to 15 frames by bees by day 55 (change of –7 frames). The colony with the VarroxSan treatment, however, increased by 5.5 frames of bees over that period (from 12 frames of bees to 17.5 frames of bees). At day 14, the colony receiving the Formic treatment had dropped in mites from 16.3% to 1.5% but rebounded to 9.4% by day 55. The colony receiving the VarroxSan treatment dropped from 14.6% to 5.5% by day 14 and dropped further to 1.9% by day 55.

Conclusion

Our results indicate that VarroxSan is a viable tool for controlling mites during the summer honey flow based on health metrics and mite control. Colonies treated with VarroxSan grew consistently over the trial period, and there was no queen loss evident. It was the only treatment group where mite infestation was below 1% at the end of the trial period, indicating consistent varroa control.

Interestingly, mite control was achieved with VarroxSan in a colony that had high mite loads (>10%) at the start of the trial. While this was only in a single case, it is quite promising and should be investigated further.

In normal practice Formic-treated colonies would have received two treatments during the study period. **The ability of VarroxSan to provide comparably effective control with only a single application indicates that this treatment would provide an economic advantage in terms of labor costs.**

Discussions

In all the trials worldwide carried out with VarroxSan under field conditions the product appeared to be completely safe for brood and adult honeybees, with no adverse reactions.

Furthermore, the efficacy of the product has been proven in all the studies submitted, with an average of efficacy of 96.1%[1].

This efficacy was obtained from colonies with an initial average prevalence of varroa on adult bees of 2.62%. This initial percentage is similar to 70% of the colonies sampled from 2017 to 2021 in North America by Pollinator Partnership and NAPPC of North American beekeepers.

It is important to point out that in all trials there were colonies with an initial varroa prevalence over 4% that ended the trial with 0%.

Uruguay:
Colony N° 4:
Initial % of varroa: 6.32%.
Final % of varroa: 0%
Colony N° 10:
Initial % of varroa: 6.78%.
Final % of varroa: 0%

Argentina:
Colony N° 4:
Initial % of varroa: 6.15%.
Final % of varroa: 0%

Greece:
Colony N° VA-5:
Initial % of varroa: 4.08%.
Final % of varroa: 0%
Colony N° VA-9:
Initial % of varroa: 4.60%.
Final % of varroa: 0%

Colonies with high initial mite loads

Having colonies with initial mite loads over 10% is extremely dangerous for the survival of the hive and a focus of reinfestation for the other hives. But having a few hives per apiary with higher initial mite load is normal.

Ensuring good efficacy is extremely important to avoid late reinfestation from these hives. The study performed in Michigan State University has shown the effectiveness of VarroxSan, cutting infestation from 14.6% to 1.9% while varroa levels in hives treated with an approved product based on Formic acid remained high (reducing from 16.3% to 9.4%).

Conclusions

Although the six field trials reports presented here were performed in different regions, beehives are not unlike greenhouses. The efficacy and safety studies can be used as a reliable indicator of the potential efficacy and safety across the United States. The conditions in our trials and those in the US are alike, so the efficacy and safety of this product should be similar.

- Side by side efficacy studies with VarroxSan show better efficacy (in some cases statistically different) than registered products tested under the same conditions.
- The effects in these independent trials are consistent and statistically significantly efficacious responses.
- Conditions in beehives are very similar regardless of the location. The studies from universities and public institutes should be applicable regardless of geographic location.
- The overall efficacy and safety of VarroxSan has been demonstrated. Due to the technology of VarroxSan to control Varroa, the activity of the product is not reliant on external environmental variables.
- Hand-mixed versions of slow release oxalic are commonly used by beekeepers, which poses a high risk for the operators, the bees and the bee product consumers. VarroxSan is a ready to use product, manufactured in a GMP facility with rigorous quality assurances that will fit perfectly into the Integrated Pest Management (IPM) regime.

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[1] Efficacy calculated using the average of 3 studies with this information (95.3% / 97.7% / 95.4% = Average 96.1%)