The Bioscan 8(1): 15-20, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EVALUATION OF ESSENTIAL OILS AND CULTURAL PRACTICES FOR THE MANAGEMENT OF *VARROA DESTRUCTOR*

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KEYWORDS
Honeybee *Apis mellifera*
Varroa destructor
Essential oils
Organic acids
Queen caging

Received on :
21.03.2012

Accepted on :
07.07.2012

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ABSTRACT
Combination of different essential oils and cultural practices were evaluated for the management of *Varroa destructor* mites infesting *Apis mellifera* colonies during different seasons of the year. Different combinations were evaluated for management of *Varroa* mites infesting *Apis mellifera* colonies. The efficacy of different combination of treatments during different seasons was in the order: February-April: Queen caging + Cinnamon oil > Queen caging + Eucalyptus oil > Queen Caging + Menthol oil > Sulphur dust. However, during, May-July, Sulphur + oxalic acid was most effective followed by Sulphur + Formic acid > Sulphur + Lactic acid and >Sulphur dust, respectively. During August-October the Formic acid + Cinnamon oil was most effective followed by >Formic acid + Eucalyptus oil > Formic acid + Menthol oil and > Sulphur dust. In November-January the efficacy of different treatments was in the order Thymol + Oxalic acid > Thymol + Formic acid > Thymol + Lactic acid >Sulphur dust. The results clearly reveal that efficacy of different treatments vary with the seasons.

INTRODUCTION

Honeybees are the most important insects that have benefited mankind for medicinal and nutritional purposes for thousand of years. Honeybees are of great economic importance to agriculture not only for honey production, but also for crop pollination. The ectoparasitic mite *Varroa destructor* (Anderson and Trueman, 2000) is considered a severe pest for honey bees causing serious losses to the beekeepers (de Jong *et al*., 1982 and Baker and Peng, 1995). This mite which feeds on haemolymph of brood and adult bees causes colony disorder, weakness, decreasing brood and deforming immature and mature bees. It also reduces colony ability to pollinate plants (De Jong *et al*., 1984). Infested colony may die or migrate resulting in economic loss and honey production (Needham, 1988). The mite can be found on adult bees, on the brood and in hive debris. In a heavy infestation, pupae may not develop into adult bees. The adults that do emerge may weigh less than healthy bees (Shimanuki and Knox, 1991). Advanced infestations cause considerable damage to honey bee populations (Baker and Peng, 1995) resulting in a reduction of the quantity and quality of honey production and pollination (Spivak, 1997). Control treatments of the mite are based on the use of acaricides, produced in especialised laboratories as well as substances of natural origin (Calderone and Spivak, 1995a; Sammataro *et al*., 1998). The repeated use of synthetics varroacides results in the development of resistance in the varroa mite to these products (Thomson *et al*., 2002). Varroa population resistant to amitraz was found in the end of the 20th century (Elzen *et al*., 1999) and mite resistance has been reported for almost all chemical substances used (Pohorecka and Bober, 2007; Spreafico *et al*., 2001; Thompson *et al*., 2002). There is current concern about contamination of bee products with synthetic substances against the varroa (Howis and Nowakowski, 2009).

It is now crucial that beekeepers have access to new control tactics that do not harm bees or contaminate hive products. Ethereal plant oils, also known as essential oils and other volatile compounds have been used to control bee mites with some success (Ariana *et al*., 2002; Calderone and Spivak, 1995b; Colin *et al*., 1997; El- Shemy, 1997; Imdorf *et al*., 1995; Ruffinengo *et al*., 2007; Sammataro *et al*., 2004). Recently, two commercial essential oil products (Apiguard® and ApiLife Var®) for *Varroa destructor* control have become available in the USA. Both products kill phoretic mites on adult bees through the slow release (Imdorf *et al*., 1999) via sublimation during routine hygienic and trophallaxis bee behaviour, of thymol and other essential oil vapours from crystals embedded in a polyacrylic acid gel matrix. Thymol has been approved by the Environmental Protection Agency as an acaricide against *V. destructor*, and no adverse effects on humans have been found (Whittington *et al*., 2000). Essential oils kill the mites when they come in contact within a few minutes and they also impair their reproduction via feeding. When varroa mites feed on larvae that contain essential oils, their reproduction is interrupted. If the oil is strong enough, the females are unable to lay eggs. If the oils are in lower concentration, eggs are layed, but development of immature mites is delayed; young mites do not reach maturity before

the bees emerge from the cell; consequently, the immature mites die (Allam *et al.,* 2003). The present investigation was aimed to determine the effective impact of some organic acids and oils in the form of modules for different seasons.

MATERIALS AND METHODS

Modules for the management of *Varroa* mites

Several organic acids and essential oils used for management of *Varroa* mites are given in Table 1. Different modules and combinations thereof were developed for management of *Varroa destructor* (Table 2) these modules were evaluated in different seasons depending upon the brood reaing condition of the colonies. Each module consisted of six treatments which included four combinations and two controls viz. water spraying and no treatment, during February to April. Three applications of each treatment were given in the beginning of each trimester of treatment modules. Pre-treatment count of mites was taken before each application. Thereafter, observations were made after 7, 14 and 21 days after treatment (DAT). For this purpose, 100 cells containing brood were examined for presence of mites.

Application methods

Oxalic Acid

Oxalic acid is applied when the colony has no brood. Any open brood in the colony is likely to be killed by oxalic acid. Therefore, two methods were used for the application of oxalic acid which included trickling and spraying (Charriere and Imdorf, 2002). For this purpose, 1 litre of acid-sugar syrup solution in the ratio of 1:1 sugar + water was prepared to which 35g of oxalic acid crystals were added and stirred gently until fully dissolved.

Trickling method

Trickling oxalic acid dihydrate seemed to be the most suitable application method, especially for large apiaries. This method was used during the presence of brood in the colonies or when brood rearing activity was in peak. The application was quick, about 1 min per hive, cost-effective and easy to conduct. The method can be used as a standard treatment due to its effectiveness, tolerability and simplicity of application. With a syringe or applicator, 5mL of solution was trickled directly onto the bees in each of the occupied bee spaces between frames in each brood box. The maximum dose was 50mL of acid solution per colony whether it is a nucleus, single or multiple brood chambered hive. Alternatively, vapourizer method could also be used using brood rearing conditions. To use the vapourizer method, all upper hive entrances and cracks have to be sealed and the main entrance should be reduced; bees may be smoked up from the bottom board and 2g of oxalic acid may be placed into the vapourizer. Vapourizer should be inserted through the bottom entrance.

Spraying method

Solutions of oxalic acid dihydrate were sprayed onto the bees on both sides of each comb and the bees resting on the hive walls; spraying was normally carried out during the broodless period.

Lactic acid (15%)

5mL of 15 per cent lactic acid was sprayed directly on each comb face using a hand or back-pack sprayer in the beginning of each treatment as described above.

Sulphur dusting

Sulphur dusting @ 1g per frame at monthly intervals was done regularly throughout the year in accordance with the module plan.

Thymol dusting

1g of thymol in powder form mixed with10-15g of wheat floor per colony was dusted on infested frames.

Formic Acid

180mL of 85% formic acid was kept in a bottle and was placed in an empty space above the brood or adjacent to the brood. The bottle was corked in such a way so as to regulate 10mL of the acid to evaporate daily.

Feeding of essential oils

Essential oils viz. menthol, cinnamon and eucalyptus were fed to the bees mixed in sugar candy prepared by mixing sugar and water 9:1 (Allam *et al*., 2004). The sugar candy was placed on the top bars of hive and the hive entrances of the test colonies were closed and the bees were not allowed to forage for next 12h so that they could feed on the sugar candy. The hive entrances were then opened to allow free foraging by bees.

RESULTS

The efficacy of different modules was evaluated during different seasons. The results obtained are as given below:

Management of mites during February to April 2006-07 and 2007-08

Each module formulated for the management of honeybee mites was comprised of six treatments as shown in the tables 3-6. Module during February-April, comprised of Queen caging + Feeding of menthol Oil; Queen caging + Feeding of Eucalyptus Oil; Queen caging + Feeding of Cinnamon Oil and Sulphur dusting alone; Water spraying and no treatment served as control. In total three applications of each treatment were given from February to April *i. e.* 1st at the beginning of February, 2nd at the beginning of March and 3rd at the beginning of April. Pre-treatment count was taken before each application. The data presented in table 3 shows that in case of Queen caging + Menthol Oil treatment, number of mites in pre-treatment count was 40.06 which after the 1st application declined to 29.25 at 7 DAT; 27.12 at 14 DAT and 25.00 at 21

Table 1: Different chemicals used in study

S.no.	Name of acricides	Concentrations (%)
1	Oxalic acid	3.5%
2	Lactic acid	15%
3	Formic acid	10 mL/day
4	Menthol oil	500ppm
5	Eucalyptus oil	500ppm
6	Cinnamon oil	500ppm
7	Thymol	500ppm
8	Sulphur	1g/frame

Table 2: Different modules for the management of mites

Module I	Feb-Apr.	Queen caging + menthol oil	Queen caging + eucalyptus oil	Queen caging+ cinnamon oil	Sulphur	Control (Water)	Control (N.T)
Module II	May-July	Sulphur + oxalic acid	Sulphur + lactic acid	Sulphur + formic acid	Sulphur	Control (Water)	Control (N.T)
Module III	Aug - Oct.	Formic acid + menthol oil	Formic acid + eucalyptus oil	Formic acid + cinnamon oil	Sulphur	Control (Water)	Control (N.T)
Module IV	Nov-Jan.	Thymol + lactic acid	Thymol + oxalic acid	Thymol + formic acid	Sulphur	Control (Water)	Control (N.T)

Where : N.T = No treatment

Table 3: Effect of different treatments in the management of mites during February-April 2006-07 and 2007-08 (Pooled)

Treatment	Pre treatment count	1st Application			Pre treatment Count	2nd Application			Pre treatment Count	3rd Application			Total percent reduction
	No of Mites/ 100 Cells	No of mites/100 cells 7DAT	14DAT	21DAT	No of mites/ 100 cells	No of mites / 100 cells 7DAT	14DAT	21DAT	No of mites/ 100 cells	No of mites /100 cells 7DAT	14DAT	21DAT	
Q.C + M.O	40.06	29.25	27.12	25.00	26.50	22.25	18.00	16.75	19.62	16.75	15.12	12.50	68.79
Q.C + E.O	41.18	26.00	23.00	19.87	26.00	17.75	15.00	12.87	19.25	16.75	12.00	9.75	76.32
Q.C + C.O	41.87	25.87	21.62	18.25	25.75	14.75	12.50	10.00	17.87	11.25	8.25	5.50	86.86
Sulphur	40.12	32.50	33.50	35.37	39.37	35.25	44.25	37.50	39.50	33.00	34.12	33.37	16.82
Control (Water)	41.25	42.75	46.00	48.25	48.25	49.00	54.25	48.12	47.62	48.37	48.25	49.75	-20.60
Control(N.T)	40.12	39.00	40.37	41.50	42.37	41.25	45.50	44.62	45.25	45.87	46.50	49.37	-23.05
C.D at 5%	1.624	2.32	1.99	2.45	2.38	2.08	2.45	2.23	2.22	1.73	2.54	2.41	

Q.C = Queen caging: M.O = Menthol Oil; E.O = Eucalyptus Oil; C.O = Cinnamon Oil

Table 4: Effect of different treatments in the management of mites during May-July 2006-07 and 2007- 08 (Pooled)

Treatment	Pre treatment count	1st Application			Pre treatment Count	2nd Application			Pre treatment Count	3rd Application			Total percent reduction
	No of mites/ 100 Cells	No of mites/100 cells 7DAT	14DAT	21DAT	No of mites/ 100 cells	No of mites/100 cells 7DAT	14DAT	21DAT	No of mites/ 100 cells	No of mites/100 cells 7DAT	14DAT	21DAT	
Sulphur + O.A	42.50	20.37	21.12	22.62	27.62	13.87	13.75	14.50	17.62	5.75	7.25	7.75	81.76
Sulphur + L.A	40.87	28.12	25.62	23.62	33.25	24.62	23.25	21.25	26.00	20.00	18.62	16.25	60.23
Sulphur + F.A	43.37	25.00	22.00	20.62	30.50	21.00	18.87	16.75	23.25	14.12	12.87	11.50	73.48
Sulphur	42.37	32.00	36.00	36.87	38.00	31.62	32.37	33.75	39.00	29.62	31.00	31.62	25.37
Control (Water)	40.75	43.75	41.12	44.25	41.62	42.75	44.12	45.12	48.50	45.62	46.87	47.50	-16.56
Control(N.T)	42.00	34.37	35.50	36.75	37.37	37.75	38.37	39.50	42.50	43.37	43.37	45.25	-07.73
CD at 5%	1.478	2.39	2.25	2.31	2.36	2.54	2.44	2.26	2.49	2.73	2.62	2.40	

O.A = Oxalic acid; L.A = Lactic acid; F.A- Formic acid; N.T = No treatment

DAT. Precount at the beginning of 2nd application was 26.50 where infestation declined to 16.75 at 21 DAT and at 3rd application it was 12.50, 21DAT, which marked an overall reduction of 68.79 percent. Similarly in case of Queen caging + Eucalyptus Oil, infestation declined from 41.18 to 26.00, 23.00 and 19.87 mites at 7, 14 and 21,DAT respectively at 1st application. However, it further declined to 9.75 at 21 DAT at 3rd application, showing 76.32 percent of total reduction. In case of Queen caging + Cinnamon Oil, number of mites declined from 41.87 to 25.87, 21.62 and 18.25 at 7, 14 and 21 DAT of the 1st application, which further declined to 5.50 at 21 DAT of the 3rd application. The overall reduction was 86.86 percent. In case of sulphur dusting alone, infestation declined from 40.12 to 32.50, 33.50 and 35.37 mites at 7, 14 and 21 DAT respectively at 1st application, which further declined to 33.37, 21 DAT at 3rd application resulting in the overall reduction of 16.82 percent, (Table 3).The studies revealed that all these four treatments were better as compared to control but were significantly different from each other.

In the module during May to July also the treatments were given at the beginning of each month. In case of Sulphur dusting + Oxalic acid at 1st application, the number of pre treatment count of mites declined from 42.50 to 20.37, 21.12 and 22.62 at 7, 14 and 21 DAT respectively (Table 4), which subsequently declined to 7.75 mites, 21 DAT at 3rd application, showing an overall reduction of 81.76 percent. In case of Sulphur dusting + Lactic acid treatment during 1st application, number of mites declined from 40.87 (pre-treatment count) to 23.62 at 21 DAT and the infestation further declined from to 21.25 and 16.25 mites at 21 DAT after 2nd and 3rd application respectively, marking an overall reduction of 60.23 percent. Similarly in case of Sulphur + formic acid application, number of mites declined from 43.37 (pre-treatment count of 43.37 mites of the 1st application to 11.50 mites at 21 DAT of the 3rd application, resulting an overall reduction73.48 percent. Sulphur dusting alone was less effective. All these treatments differed significantly but were superior over control.

The module during August to October (Table 5) was comprised of Formic acid + Menthol oil; Formic acid + Eucalyptus oil; Formic acid + Cinnamon oil; Sulphur dusting alone, water spraying and no treatment. In case of Formic acid (fumigation) + Menthol oil (feeding) the number decreased from 45.12 to 25.37, 22.87 and 20.75 mites, 7, 14 and 21DAT respectively, after 1st application. The number further declined to 16.50

Table 5: Effect of Different treatments in the management of mites during August-October 2006- 07 and 2007-08 (Pooled)

Treatment	Pre treatment count	1st Application			Pre treatment Count	2nd Application			Pre treatment Count	3rd Application			Total percent reduction
	No of mites / 100 cells	No of mites / 100 cells			No of mites / 100 cells	No of mites / 100 cells			No of mites / 100 cells	No of mites /100 cells			
		7 DAT	14 DAT	21 DAT		7 DAT	14 DAT	21 DAT		7 DAT	14 DAT	21 DAT	
Formic Acid +M.O	45.12	25.37	22.87	20.75	34.62	21.37	17.87	16.50	29.00	16.12	14.00	13.12	70.92
Formic Acid+ E.O	45.37	22.25	22.87	18.12	30.00	18.62	14.75	13.75	26.62	12.62	11.00	9.12	79.89
Formic Acid + C.O	43.62	19.25	17.37	15.87	29.12	15.37	11.75	9.62	16.87	7.37	6.00	4.00	90.82
Sulphur	47.00	46.62	47.37	49.12	48.37	40.37	42.75	43.62	43.25	37.75	37.12	40.00	14.89
Control (Water)	44.50	42.37	44.37	45.25	45.37	47.75	49.00	50.37	51.12	52.00	53.12	52.50	-17.97
Control. (N.T)	45.62	44.00	43.62	46.25	46.37	46.62	47.00	47.50	50.50	49.25	50.37	51.87	-13.70

M.O = Menthol Oil; E.O = Eucalyptus Oil; C.O = Cinnamon Oil; N.T = No Treatment

Table 6: Effect of Different treatments in the management of mites during November-January, 2006-07 and 2007- 08 (Pooled)

Treatment	Pre treatment Count	1st Application			Pre treatment Count	2nd Application			Pre treatment Count	3rd Application			Total percent reduction
	No of mites / 100 cells	No of mites / 100 cells			No of mites / 100 cells	No of mites / 100 cells			No of mites / 100 cells	No of mites /100 cells			
		7DAT	14DAT	21DAT		7 DAT	14DAT	21DAT		7DAT	14DAT	21DAT	
Thymol+L.A	37.12	28.62	26.00	23.37	28.37	21.62	20.00	18.37	23.62	17.62	15.00	13.87	62.63
Thymol+O.A	38.62	17.25	18.25	19.50	26.00	13.12	14.50	15.62	19.50	7.12	7.87	9.37	75.73
Thymol+F.A	37.50	19.62	18.25	17.12	26.12	18.12	16.50	15.00	19.50	17.00	14.25	11.75	68.66
Sulphur	38.57	37.25	37.87	38.62	38.12	32.75	34.00	35.37	38.75	31.37	32.00	33.50	13.14
Control(Water)	37.50	39.12	39.25	40.62	41.00	41.50	44.12	44.50	41.12	41.87	41.37	42.00	-12.00
Control. (N.T)	38.25	32.87	33.87	35.75	35.62	37.37	38.75	39.87	39.00	41.00	43.50	46.75	-20.26
CD at 5 %	1.178	2.11	2.15	1.89	2.66	1.95	2.12	2.25	2.02	2.49	2.38	1.92	

L.A = Lactic Acid; O.A = Oxalic Acid;F.A = Formic Acid; N.T = No Treatment.

and 13.12 mites 21 DAT at second and third application, respectively (Table 3). In case of Formic acid + Eucalyptus oil, the number during the 1st application decreased from 45.37 to 22.25, 22.87 and 18.12 mites respectively, after 7, 14 and 21 DAT. Infestation further declined to 13.75 and 9.12 mites at 21 DAT respectively during 2nd and 3rd application, showing total percent reduction of 79.89. Similarly, in case of Formic acid + Cinnamon oil, the infestation of mites declined from 43.62 (pre treatment count) to 19.25, 17.37 and 15.87 mites after 7, 14 and 21 DAT of the first application respectively. However the number further declined to 9.62 and 4.00 mites after 21 DAT respectively, during 2nd and 3rd application measuring a reduction of 90.82 percent. Sulphur dusting was less effective and the number of mite declined during 1st application from 47.00 to 49.12, 21 DAT. The decline of mites further reached to 40.00, 21 DAT at 3rd application, showing total percent reduction of 14.89. A comparative examination revealed that Formic acid + Cinnamon oil was most effective followed by Formic acid + Eucalyptus oil, Formic acid + Menthol oil and sulphur dusting, respectively.

The module during November to January (Table 6) was comprised of Thymol + Lactic acid; Thymol + Oxalic acid; Thymol + Formic acid; sulphur dusting water spray and no treatment. During this period also, three applications of different treatments, one at the beginning of each month was given. The data presented in Table 4 reveal that Thymol dusting + Lactic acid spraying suppressed the mite population from 37.12 mites (pre-treatment count) to 28.62, 26.00 and 23.37 mites at 7, 14 and 21 DAT, after 1st application and reached up to 18.37 and 13.87 mites 21 DAT respectively, after 2nd and 3rd application showing overall reduction of 62.63 percent. Similarly, Thymol + Oxalic acid treatment resulted in reduction in population of mites during 1st application from 38.62 mites to 17.25, 18.25 and 19.50 mites at 7, 14 and 21 DAT and again the number of mites reached up to 15.62 and 9.37, 21 DAT respectively, of the 2nd and 3rd application pointing on overall reduction of 75.73 percent. In case of Thymol + Formic acid treatment the number of mites declined from pre treatment count of 37.50 mites to 19.62, 18.25 and 17.12 respectively at 7, 14 and 21 DAT of the 1st application. The number of mites further declined to 11.75 mites, 21 DAT of the 3rd application, resulting in an overall reduction of 68.66 percent. In case of Sulphur dusting alone, there was no significant reduction in the number of mites and was at par with the control. Of the different treatments, Thymol + Oxalic acid were found to be the most superior followed by Thymol + Formic acid and Thymol + Lactic acid, respectively. Briefly the results revealed the most effective treatment during each module as given below:

February-April = Queen caging + Cinnamon oil.

May-July = Sulphur + oxalic acid.

August-October = Formic acid + Cinnamon oil.

November-January = Thymol + Oxalic acid.

DISCUSSION

The most common means of controlling *Varroa* has been though the use of synthetic acaricides applied in formulated plastic strips to infested colonies. Chemical control recommendations are being changed frequently as new products are developed and problems with older treatments become evident (De Jong, 1997). The mite is highly resistant to chemical treatments; therefore, even if necessary, the repeated applications of same chemical may be avoided**.** Essential oils hold good alternatives for control programs targeting *Varroa* mites. Several essential oils have shown acaricidal activity in screening tests. Essential oils are highly volatile terpenes and phenolic compounds, which have an intense aroma (Imdorf *et al*., 1999). Essential oils are byproducts of the secondary metabolism of certain plants. Plants have evolved the potential use of essential oils to control *Varroa* mites which was reported by several authors (Mautz, 1982; Rittter and Hoppe, 1986; Colin 1990; Imdorf, 1995; Amrine *et al*., 1996; Hoppe and Ritter, 1997; Bogdanov *et al*., 1998; Sammataro *et al*., 1998; Murillo–Yepes, 1998). Treatments with essential oils represent a potentially superior control for *Varroa* mites. Because of their origin and mode of action, it is possible that such compounds are more easily degraded, more specific and less susceptible to the production of resistance than synthetic pesticides currently used (Gerard *et al*., 1997). Thymol tends to be well tolerated by honeybees. Calderone and Spivak (1995) found that a blend of thymol, eucalyptus oil, menthol and camphor caused average mite mortality of 96.7 per cent. Calderone *et al*. (1997) calculated a mite mortality of 56.4 per cent and 49.1 per cent in colonies treated with different applications of thymol and cineol. The obtained data are agreement with the finding of Eguaras *et al*. (1996) and Allam *et al*. (2003), they found that formic acid killed 91.7% of the varroa mites and honey bee colonies became more active with large number of worker brood area were detected. Treatments with organic acids are considered good tools for fighting the mite in several countries. Studies in Germany have shown that 98 per cent mite mortality can be achieved by placing 1-2 absorbent pads containing formic acid on the bottom of the hive (Wachendorfer *et al*., 1985). The pads must be in place for 4 days and the treatment repeated three times (Delaplane, 1997). Radetzki (1994) demonstrated that aerosol treatments with 3 per cent oxalic acid on bees infested with adult mites produced 97.3 per cent mite mortality.

Hoppe (1990) studied the acaricidal effect and bee compatibility of 55 different essential oils and found that twenty four essential oils produced mite mortality greater than 90 per cent after 48h of evaporation. Three oils produced the same effect by topical application. Only nine of the 55 oils produced bee mortality less than 10 per cent. On the contrary, 96.8 per cent mite mortality can be achieved using the essential oil thymol. Apilife VAR®, a product containing 76 per cent thymol, accomplishes 97 per cent mite mortality and is currently used in Italy (Delaplane, 1997). In earlier studies, Hoppe (1990) studied that acaricidal effect of 55 different essential oils and found that 24 essential oils produced mite mortality greater than 90 per cent after 48 h of evaporation.

ACKNOWLEDGEMENTS

The authors wish to thank SKUAST, Jammu authorities for laboratory and field facilities. Dr Denis L Anderson for identification of the mites.

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